SUPPLEMENTARY MATERIAL

Nucleosomal DNA accessibility governed by the dimer/tetramer interface

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1. Additional Materials and Methods

1.1 Samples

Mononucleosomes were reconstituted on DNA fragments containing the SELEX generated 601 positioning sequence in the center (1) and modified or unmodified recombinant *X.laevis* histones. The SELEX generated 601 positioning sequence was used for all our experiments, to ensure a uniform nucleosome positioning. The fragments (170 bp unless stated otherwise) were prepared by PCR (2), purified with HPLC (ion exchange column, Gen-Pax FAX, Waters) and concentrated by ethanol precipitation. Fluorescent labels on the DNA (alexa 488 and alexa 594) were introduced using fluorescent primers purchased from IBA (IBA GmbH). Different fluorophore positions were chosen in order to label different parts of the nucleosomal DNA namely three internal sites (+42, -52, -15 relative to the center of the positioning sequence) within the nucleosome (see main text Fig. 2A) (2,3).

Histones were modified prior to octamer refolding. Alexa 488-maleimide was added to histone mutants H2B T112C (4) and H4 E63C (5) in unfolding buffer (7M guanidinium hydrochloride, 20mM Tris-HCl, 1 mM TCEP, pH 7.15) in several small aliquots to a final molar ratio of 4:1. The reaction was allowed to proceed for 8 hours at 4°C and quenched with an excess of ß-mercaptoethanol.

Octamers were refolded by mixing equimolar amounts of recombinant *Xenopus laevis* H2A, H2B, H3 and H4 which have been incubated in unfolding buffer for 90 minutes, and dialyzing against refolding buffer (10 mM Tris-HCl, 0.1 mM EDTA, 2 M NaCl, 5 mM ß-mercaptoethanol, pH 7.5) over night (6,7). Fluorescently labeled octamers were prepared analogously; the specific amount of wt protein was substituted by the corresponding amount labeled histone which was used without prior purification. After dialysis improperly refolded complexes and free fluorophores were separated by FPLC (Superdex 200 HR 10/10, GE Healthcare).

Since there are two copies of each histone in a nucleosome, specific labeling of one subunit within the octamer is not possible. However, for the ease of evaluation of our single molecule fluorescence data, two fluorophores on one octamer must be avoided. In FCS experiments these octamers do not, due to their higher molecular brightness, contribute in direct proportion to the autocorrelation function. Furthermore, the presence of two donor or two acceptor molecules affects the measurement of the proximity ratio in FRET experiments. We therefore developed the following labeling stategy: We purposely labeled only 10 % of the particular histone using the donor fluorophore alexa 488. After octamer refolding and nucleosome reconstitution, 80 % of the nucleosomes were not labeled with a donor fluorophore and were either not detected in the experiment (FCS) or could be excluded during data analysis (spFRET). A negligible number of nucleosomes (1 %) carried two donor fluorophores. 20 % of the nucleosomes, respectively 95 % of all nucleosomes that were used for data evaluation, carried one donor fluorophore and were suited for FCS and spFRET measurements.
Nucleosome reconstitution was carried out by mixing 4 µmol DNA with 6 to 7 µmol octamer in 100 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5) with 2 M NaCl. The sample was filled into a small dialysis tube which in turn was placed into a second dialysis tube containing 15 ml of TE with 2 M NaCl. This was dialyzed against 1 l of TE with 0.1 M NaCl for 5 hours at 4°C. Then the small dialysis tube was placed in 700 ml of TE with 5 mM NaCl over night. The quality of reconstituted nucleosomes was determined with 6 % native PAGE (8) (see Fig. S1A). Only nucleosomes without free DNA were used for further experiments. Using a Cary dual beam absorption spectrophotometer (Varian, Mulgrave, Australia) the concentration was determined and the absence of aggregates was verified. The fluorescence

anisotropy (r) of the fluorophores in nucleosomes was measured using a commercial fluorimeter (SLM 8100, SLM-Aminco, Urbana, IL) (9).

1.2 spFRET

An inverted microscope Olympus IX70 supplemented with confocal excitation and detection optics of our own construction was used as described (2). Donor excitation was done with a continuous wave Ar/Kr laser (Melles Griot) at 488 nm at 100 µW. The fluorescence emission then passes a dichroic mirror (BrightLine FF498/581-Di01-22D, Semrock) and is split into two spectral windows defined by a beam splitter (600dcxr, Omega Optical) and appropriate filters (520DF40 for alexa 488 emission, 610ALP for alexa 594 emission, both Omega Optical). The single-photon data stream was collected from two avalanche photodiodes (APD, Perkin Elmer Optoelectronics). Data was read out by a TCSPC board (TimeHarp200, Picoquant GmbH). Software developed in our group was used to extract single molecule events with given constrains (at least 15 photons/ms, a total of at least 60 photons, maximal 120 µsec interphoton time) and to calculate the proximity ratio P(t) from the photon counts in both channels $N_{D/A}$ (see main text *Materials and Methods, equation 1*). This ratio is closely related to the FRET efficiency and provides information on the distance between fluorophores while still containing instrument specific parameters (10). A detailed description of the program can be found in (10). In separate measurements nucleosomes labeled only with donor fluorophore were used to determine the cross talk of donor emission in the acceptor detection channel (spectral cross talk). DNA labeled with donor and acceptor at a distance that did not allow energy transfer was used to determine direct excitation of the acceptor fluorophore at the donor wavelength. Protein and DNA labeled samples were corrected for background level and spectral cross talk. In samples where the donor, as well as the acceptor, fluorophore were attached to the DNA, both fluorophores always pass the confocal volume at the same time and therefore these samples have been further corrected for the direct acceptor excitation rate. Consequently, in these samples particles missing the acceptor fluorophore display a proximity ratio below zero.

In spFRET experiments the concentration of labeled nucleosomes is critical. While low concentrations of FRET labeled particles require long measurements, in high concentrations two or more particles might be detected simultaneously. 50 pM had proven to be a good compromise (2). Since only 20 % of all nucleosomes were FRET labeled, the total nucleosome concentration was set at 250 pM. For DNA^{+42}-DNA^{-52} nucleosomes, all nucleosomes carried a donor and an acceptor fluorophore. To obtain the same overall nucleosome concentration at the same donor fluorophore concentration, a corresponding amount of unlabeled nuclesomes or unlabeled DNA was added for spFRET experiments.

The proximity ratio distribution was illustrated in histograms for every experiment. An example histogram is depicted in Fig. S2. In most cases, at least two populations, the FRET and the NoFRET population, could be discriminated. Their quantification was complicated in some conditions by the appearance of broad populations or very small populations. We therefore used the following approach for the quantification of the FRET population: If only one population could be fitted stably, we calculated the ratio of the integral over this population to the total number of bursts in this histogram using Matlab 2008a. The approach was validated when both populations could be fitted and independent of the population that was fitted the same results for the ratios were obtained. A population centered at P(t)=1 arises from the direct excitation of particles that contain only an acceptor fluorophore. These bursts can be distinguished from bursts arising from particles with donor and acceptor fluorophores by their low photon rates, and were neglected for further analysis. Further data evaluation (sigmoidal and linear curve fitting) was carried out using IgorPro 4.07 (WaveMetrics, USA).

Experiments were carried out in 384-Well Glass Bottom Microplates (Greiner Bio-One) which were treated with SigmaCoat (Sigma). For the analysis of nucleosome disassembly the nucleosome stock solution (~ 300 nM) was diluted to a final concentration of 250 pM in spTE buffer (TE buffer supplemented with 1 mM VitaminC, 0.02 % Nonidet P40) containing between 150 and 1200 mM NaCl and incubated for 90 minutes prior to 14 minutes measurement. For the analysis of assembly, nucleosomes were reconstituted from DNA and histone octamers at 4°C and in a salt step dialysis, which allowed us to specify [NaCl] at the end of each step. The NaCl concentration was reduced from 2000 mM to 100 mM NaCl over the course of 5 hours in 9 steps, each step taking 30 minutes. At the end of each step an aliquot of the sample was diluted approximately 1000 fold (from ~ 300 nM to 250 pM) in spFRET buffer with the corresponding NaCl concentration for measurement (15 minutes for 1000 mM and 800 mM, 25 minutes for lower NaCl concentrations). After the experiment, the efficiency of the reconstitution was assayed using native PAGE.

For H2A-H2B exchange experiments, nucleosomes labeled with alexa 488 at H2B T112C and nucleosomes labeled with alexa 594 at DNA^{-52} were mixed at equimolar amounts in spFRET buffer containing either 760 mM or 380 mM NaCl at a total nucleosome concentration of 500 pM. After 90 minutes incubation, half of the samples were analyzed using spFRET, the other half were diluted to 250 pM total nucleosome concentration and 380 mM NaCl, incubated for further 20 minutes, and analyzed in spFRET.

1.3 FCS

Autocorrelation experiments were performed in the spFRET setup while the signal from the detectors was read out by an ALV5000/E autocorrelator (ALV GmbH). The laser power was set to 20 µW to reduce photobleaching effects and the triplet fraction. Nucleosomes were used that contained only a donor fluorophore, either on the H2B (T112C) or the H4 (E63C) or the DNA, in order to avoid variances in molecular brightness due to FRET or cross talk of the acceptor fluorophore. Experiments for nucleosome dissociation and reconstitution were carried out analogously to spFRET experiments, only the nucleosome concentration was increased to 20 nM during the measurements. Furthermore, we used 210 bp DNA fragments for the analysis of nucleosome assembly in order to maximize the difference in diffusion coefficients between histones and histone-DNA complexes. The autocorrelation curves were fitted by a normal diffusion model, as described in (11) using the program Quickfit (12).To account for effects of NaCl concentration on buffer viscosity and refractive index, the diffusion coefficient was determined relative to that of free fluorophore alexa 488.

2. Supporting Results

2.1 Characterization of fluorescent nucleosomes

For our single molecule fluorescence analysis we prepared nucleosomes that carried a label either on H2B (T112C), or on H4 (E63C) or on the DNA (+42, -52 or -15). The positions were chosen as they are known not have an impact on the nucleosome structure (2-5). Comparing all nucleosome constructs in native PAGE which is a very sensitive measure for the structural integrity of nucleosomes and DNA positioning on the octamer (5) revealed that all nucleosomes displayed the same mobility (Fig. S1A).

To validate our nucleosome constructs for FRET measurements, we had to verify that the fluorophores are not restricted by partial burial or interactions with neighboring hydrophobic surfaces. We measured the anisotropy r, a value for the rotational freedom of the fluorophore

for every construct (9). For all fluorophores in the nucleosome constructs the anisotropy lies below 0.2. We can therefore assume that the donor and acceptor move freely and that energy transfer is rapidly averaged over all available fluorophore orientations.

With the goal to exclude photophysical effects of the fluorophores at elevated [NaCl], we measured the count rate per particle in FCS experiments at different salt concentrations, which would reveal a change in the quantum yield or a spectral shift of the fluorophores. Neither alexa 488 (fluorophore attached to the octamer within the nucleosome or to the DNA) nor alexa 594 (fluorophore attached to the DNA) showed a change in the count rate per particle on increasing salt concentration (Fig. S1B). Therefore we concluded that an eventual spectral shift of the fluorophore emission or a change in quantum yield is negligible.

2.2 Interfluorophore distances

The distances between the labeled positions within the nucleosome were derived from the crystal structure (see Table S1). They provide a rough estimate for the interfluorophore distance neglecting the linker between nucleosome and fluorophore. If one label is attached to a histone, two distances are given, depending on which of the two identical histones is labeled.

spFRET allows us to unravel subpopulations that differ in the interfluorophore distance. Consequently, the FRET population for histone-labeled samples should be detected as two Gaussian populations, while the FRET population for only DNA- labeled nucleosomes should be detected as a single Gaussian population.

This is consistent with what we find for H4-DNA^{-15}, H4-DNA^{-52} and DNA^{+42}-DNA^{-52}. However, the FRET population of H2B-DNA^{-52} shows a single Gaussian distribution. Furthermore, in H2B-DNA^{-15} the distances between the label on the DNA and on H2B are similar, so that we would expect a single Gaussian distribution, while we detect a double Gaussian distribution for the FRET population. However, this simple estimate does not consider the possibility that the fluorophores could assume preferred positions that might differ slightly from the estimates given above.

2.3 Comparison of nucleosomes and free H2A-H2B in FCS

We have demonstrated the sequential dissociation of H2A-H2B and $(H3\text{-}H4)_2$ upon increasing [NaCl] (see main text *Results 3.1*). To identify the diffusing particle in the sample, we compared the relative diffusion coefficient of the H2B labeled sample to that of free H2A-H2B (see Table S2).

While at 300 mM NaCl the diffusion coefficient of free H2A-H2B was lower than the one of the H2B labeled nucleosome sample, the diffusion coefficient was nearly identical at 1100 mM NaCl, strongly indicating that the diffusing particle in the experiment at high salt is free H2A-H2B that dissociated from the nucleosome.

2.4 H2A-H2B exchange between nucleosomes

To confirm H2A-H2B dissociation, we wanted to demonstrate H2A-H2B exchange between nucleosomes. For this purpose we performed the following spFRET experiments: nucleosomes were prepared, which carried, either, only donor fluorophores on the H2B or only acceptor fluorophores on the DNA (DNA^{-52}). These nucleosomes were mixed

stoichimetrically, and then incubated at 760 mM NaCl to destabilize the H2A-H2B/DNA interaction (corresponding to $c_{1/2}$ for H2A-H2B dissociation, see main text *Results 3.2*). Salt was then reduced by a one-fold dilution to 380 mM NaCl. From this assay, we observe the appearance of a FRET population in the spFRET analysis (Fig. S3A and S3B). The mean FRET value of this population (x_0 = 0.52±0.01) was the same as those which were assembled as double labeled nucleosomes H2B-DNA^{-52} (x_0=0.53±0.01). We conclude that H2A-H2B has dissociated from nucleosomes at 760 mM NaCl and upon lowering [NaCl] bound to different histone-DNA complexes (label with acceptor at the DNA) in the same position as in intact nucleosomes (Fig. S3C). As a control, nucleosomes incubated under 380 mM NaCl show no detectable FRET population, indicating that nucleosome destabilization at elevated [NaCl] is necessary to induce H2A-H2B dissociation and exchange (Fig. S3D).

2.5 Analysis of FRET distributions

In the main text we analyzed the increase of the NoFRET population at the cost of the FRET population. Structural changes within the nucleosome will however also cause changes in the proximity ratio distribution resulting in shifts of the FRET population. Therefore in the following we analyzed changes in the proximity ratio distribution to reveal additional insights into minor conformational changes within the nucleosome (see main text, Fig. 4B). The DNA^{+42}-DNA^{-52} construct shows a second population with higher proximity ratios appearing above 150 mM NaCl while the total number of FRET bursts does not change. Upon elevating [NaCl] its proportion increases compared to the FRET population with lower proximity ratios until 800 mM NaCl, at which point both populations were approximately equal. This indicates that part of the nucleosomes adopts a conformation with a shorter interfluorophore distance, which might correspond to state IV and state V (see main text, Fig. 1) in the disassembly pathway.

The H2B-DNA^{-15} and H2B-DNA^{-52} constructs show bursts with lower proximity ratios arising at 600 mM (see main text, Fig. 4B), indicating that the distance between the labeled positions has increased, while energy transfer still occurs. This can be interpreted as a general opening of nucleosomes in part of the population, increasing the donor-acceptor distance. The H4-DNA^{-52} construct also showed bursts with lower proximity ratios appearing near 800 mM NaCl. We propose that this is the point at which the DNA begins to unroll partially from the histones in the (H3-H4)$_2$-DNA complex and, consequently, the distance between (H3-H4)$_2$ and DNA^{-52} increases. For H4-DNA^{-15}, however, the shape of the FRET population does not change until 1000 mM NaCl. Thus, (H3-H4)$_2$ is firmly bound to its position on the DNA dyad axis until it is dissociated.

Alternatively, one might postulate that the αC helix of H2B (to which the fluorophore is attached) could move independently in response to salt resulting in the observed intermediate state. However, the structure of the nucleosome and the structure of the histone octamer at 2 M salt are virtually identical. While we cannot exclude that the helix shifts at intermediate salt concentrations, it is highly unlikely. The helix is extremely well ordered and cemented to the underlying histone fold regions by numerous hydrophobic interactions (Fig. S6A; in blue are H2A - Y57, Y50; H2B - H106, Y118).

In addition, flipping of H2B αC cannot result in the observed loss of FRET between H2B/DNA^{-15}. Based on measurements from x-ray crystallographic data, the dye to dye distance between H2B and DNA^{-15} is ~ 56 Å. If we allow the αC of H2B to be mobile around a hinge point in the connecting loop (Fig. S6B), we predict a maximum dye to dye distance of

64 Å, well within our range of measurement. This small distance change would be apparent in a small decrease in proximity ratio, not the large loss of FRET observed in our studies.

2.6 Nucleosome assembly analyzed by FCS

To our knowledge, nucleosome disassembly and assembly have never been probed using the same experimental approach. To test the reversibility of the disassembly process described above, we monitored nucleosome assembly using the same FCS method as for the analysis of the disassembly. Further investigation of the reversibility was done using spFRET (see main text, *Results 3.3*).

We reconstituted nucleosomes (fluorescently labeled either on H2B, H4, or DNA as for the dissociation study) from octamer and DNA by reducing the salt concentration in a stepwise manner from 2000 to 300 mM NaCl and measured the diffusion coefficients of the labeled subunits (H2A-H2B, $(H3\text{-}H4)_2$ or DNA) at each step (Fig. S4). For samples containing labeled DNA, the diffusion coefficient increased with decreasing [NaCl], indicating that the DNA adopts a more compact form, consistent with nucleosome formation. The diffusion coefficient for H4-labeled samples decreased starting at 1800 mM NaCl, whereas the diffusion coefficient of H2B-labeled samples decreased starting at 1400 mM NaCl. This confirms that even if the reconstitution is started with histone octamer, $(H3\text{-}H4)_2$ binds the DNA at higher ionic strength than H2A-H2B (13).

2.7 Effect of nucleosome concentration

Nucleosome concentration is known to influence nucleosome stability significantly (2,14). As a necessity the nucleosome concentrations differ between our experiments. While spFRET experiments have to be performed at conditions where maximally one nucleosome is in the focus at a time (250pM), FCS experiments have to be performed at a higher concentration for a good signal to noise ratio (20 nM).

Since we were able to investigate disassembly experiments at both 20 nM and 250 pM nucleosome concentrations, we could image under equilibrium conditions (after 90 minutes incubation time). However, for effective assembly we need significantly higher nucleosome concentrations because at low sample concentrations, effective assembly is impeded by effects such as adsorption to the dialysis tube. Therefore, assembly experiments were carried out at 300 nM nucleosome concentration. For the analysis with FCS and spFRET, samples were diluted to experimental concentrations and then were analyzed immediately after dilution in order to be as close as possible to the state of the sample during the reconstitution. While our results show that the structural changes detected in FCS and spFRET during assembly and disassembly followed the same sequence, they occurred at different [NaCl]. In the following we show that these differences can be explained by the differences in nucleosome concentration and incubation time.

FCS (20 nM) vs. spFRET (250 pM)

By adding unlabeled nucleosomes we increased the nucleosome concentration from 250 pM to 20 nM in a spFRET experiment. As a representative example, we used DNA^{+42}-DNA^{-52} nucleosomes and quantified the FRET fraction after incubation at 860 mM NaCl. We chose this [NaCl] because it is the $c_{1/2}$ for this construct at 250 pM. While at 250 pM nucleosome concentration, 50 % of nucleosomes displayed FRET, at 20 nM nucleosome concentration 80 % of the nucleosomes displayed FRET (Fig. S5A), confirming that increasing the nucleosome

concentration increases nucleosome stability. We suggest that the observed discrepancy between FCS and spFRET is due to this difference in nucleosome concentration.

Ideally, we would like to perform dissociation experiments at the same nucleosome concentrations as those performed for FCS. However, the addition of unlabeled nucleosomes can not be used in a spFRET experiment to increase the nucleosome concentration when donor and acceptor fluorophore are attached to different subunits of the nucleosome. Subunits can exchange between the labeled and unlabeled nucleosomes. Considering the large surplus of unlabled nucleosomes, it is likely that single labeled nucleosomes arise. Consequently, the loss of FRET can not necessarily be linked to nucleosome disassembly any more.

Disassembly vs. Assembly

We analyzed the FRET fraction over the course of 30 minutes after dilution of the sample at 1000 mM NaCl. As an example for all constructs, we used H4-DNA^{-15} nucleosomes. Fig. S5B shows that the FRET fraction decays exponentially over time. We suggest that the observed discrepancy between assembly and disassembly arises is due to the differences in incubation time.

2.8 Number of ion pairs

The correlation of nucleosome disassembly and [NaCl] can be used to determine the number of ion pairs (m) between histones (H) and DNA following a thermodynamic analysis from Record et al. (15)(see Eq. 1).

$$-\frac{\partial \log K}{\partial \log[Na^+]} = -\frac{\partial \log\left(\frac{[H-DNA]}{[H][DNA]}\right)}{\partial \log[Na^+]} = 0.88 \cdot m' \qquad [1]$$

The concentration of the intact histone-DNA complex is given by the FRET fraction, whereas the concentration of dissociated histone [H] and the remaining nucleosome fragment [DNA] are identical and given by the NoFRET fraction. The factor 0.88 arises from the fraction of counterions thermodynamically bound to DNA per phosphate (15).

The number of ion pairs that are involved in interaction of the two binding partners can be calculated from linear curve fitting of the logarithm of K as a function of the logarithm of [NaCl] (see main text, Fig. 7A). The results from these fits are reported in Table S3.

The loss of FRET in H2B-DNA^{-15} was not assigned to a dissociation event, but to the opening of the nucleosome structure. The salt dependence of the opening-closing transition can be isolated from the other transitions by the following procedure. Suppose we start with the equilibrium below, where N is the closed, N_O the open and N_{diss} the partly dissociated nucleosome (see main text, Fig. 1, I corresponds to closed state, IV to open state and V as well as VI to dissociated states), H_D is the dissociated H2A/H2B dimer, M^+ monovalent metal ions of which x are bound on the first step and y on the second step:

$$N + (x+y)M^+ \overset{K_1}{\leftrightarrow} N_O + yM^+ \overset{K_2}{\leftrightarrow} N_{diss} + H_D \qquad [2]$$

with K1, K2 defined as

$$K_1 = \frac{[N_o]}{[N][M^+]^x} \qquad K_2 = \frac{[N_{diss}][H_D]}{[N_o][M^+]^y} \qquad [3]$$

The relative amount of the FRET population for the first transition, i.e. between H2B and DNA^{-15}, is the ratio of the closed state to all (closed + open + dissociated) states:

$$F_1 = \frac{[N]}{[N]+[N_O]+[N_{diss}]} \qquad [4]$$

and likewise for the second transition from the open to the first dissociated state, i.e. between H2B and DNA^{-52}:

$$F_2 = \frac{[N]+[N_O]}{[N]+[N_O]+[N_{diss}]} \qquad [5]$$

From eqs. [4] and [5], we can easily express the ratio of the open to closed state from the FRET populations F_1 and F_2 :

$$\frac{[N_O]}{[N]} = \frac{F_2}{F_1} - 1 \qquad [6]$$

With [3] it then follows that

$$\log\left(\frac{F_2}{F_1} - 1\right) = \log K_1 + x \log[M^+] \qquad [7]$$

and plotting $\log(F_2/F_1-1)$ against the logarithm of the salt concentration will give an estimate of the number of ion pairs involved in the opening transition (see main text, Fig. 7B and Table S3).

3. References

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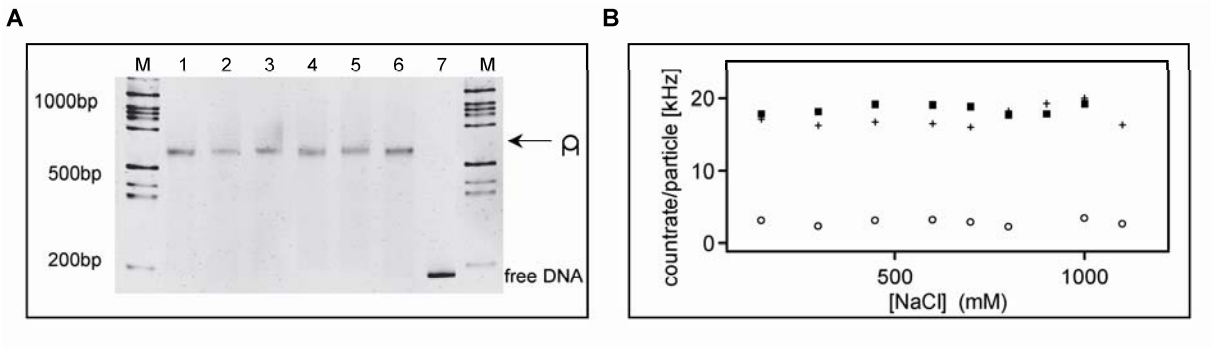
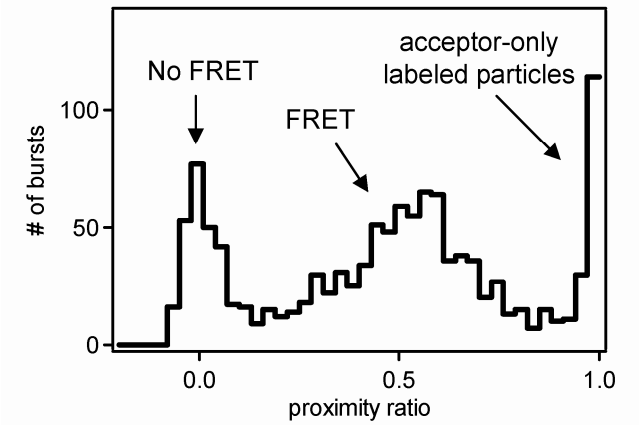
4. Figures

Figure S1



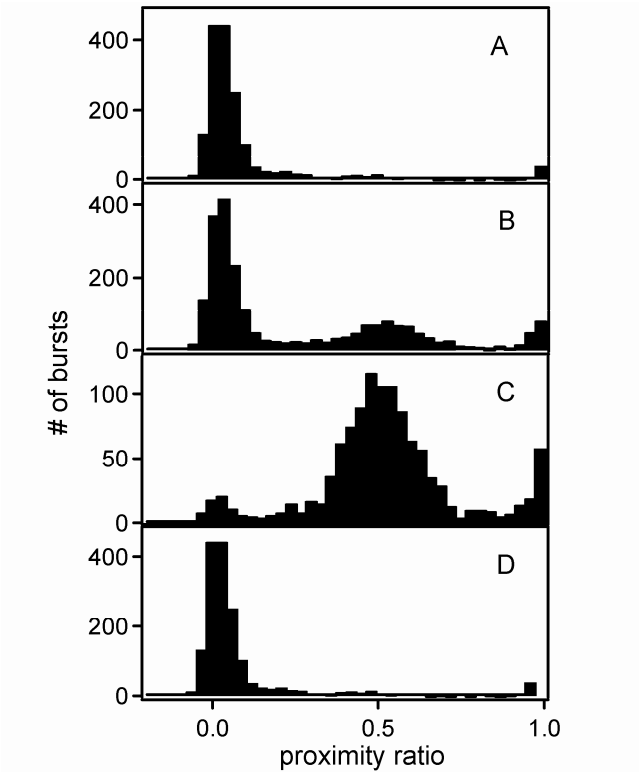
Controls for the influence of the labeling on the nucleosome structure and the salt effect on the dyes
(A) Native PAGE of all nucleosome samples used in the spFRET experiments:
(polyacrylamide 6 % in TBE buffer, samples were stained with ethidium bromide), Lane M: 100 bp Molecular Ruler (Bioron), lane 1-6: H2B-DNA^{-52}, H4-DNA^{-52}, DNA^{+42}-DNA^{-52}, H2B-DNA^{-15}, H4-DNA^{-15} and unlabeled nucleosomes respectively, lane 7: 170 bp DNA. Nucleosome samples do not contain free DNA. The presence of the fluorophores does not affect the electrophoretic mobility.
(B) Count rate per particles as a function of [NaCl] measured in FCS: Nucleosomes were labeled with alexa 488 at the octamer (H2B, cross), DNA was labeled with alexa 488 (square) or alexa 594 (circle). Data points are derived from 10 measurements. The experimental error is estimated to be less than 2 kHz. For clearness of representation, error bars are not depicted. No change in the count rate per particle was detected. We therefore concluded that an eventual spectral shift of the fluorophore emission or a change in quantum yield is negligible.

Figure S2



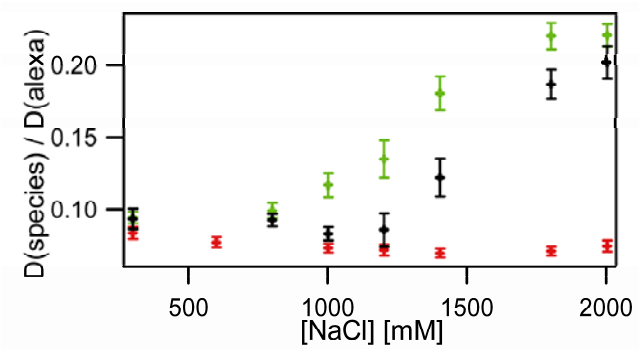
Example for a proximity ratio histogram from spFRET measurement
Three populations can be discriminated:
The population centered at 0 arises from events that do not show energy transfer, therefore referred to as NoFRET population. NoFRET bursts indicate that either the subunit carrying the acceptor fluorophore has dissociated or that due to structural changes the donor-acceptor distance within the particle has increased above the distance for energy transfer. Also nucleosomes that are missing an acceptor fluorophore, due incomplete labeling or acceptor bleaching cause NoFRET bursts.
Bursts with medium proximity ratios denote that the dyes are in close proximity, so that energy transfer can occur, therefore referred to as FRET population. A narrow distribution indicates that the dyes adopt a specific distance as it is the case if the labeled subunits are firmly bound to one another. Broader FRET populations indicate that the distances between the two dyes vary over a wide range within the sample.
The population centered at 1 is a consequence of our under labeling strategy for the histone octamer. Histone-labeled samples contain a high number of acceptor-only labeled nucleosomes. These nucleosomes can be directly excited and appear in the histogram as bursts with high proximity ratios since they do not show donor emission. Due to their low photon rates, they can be distinguished from bursts with high energy transfer and are neglected for further analysis.

Figure S3



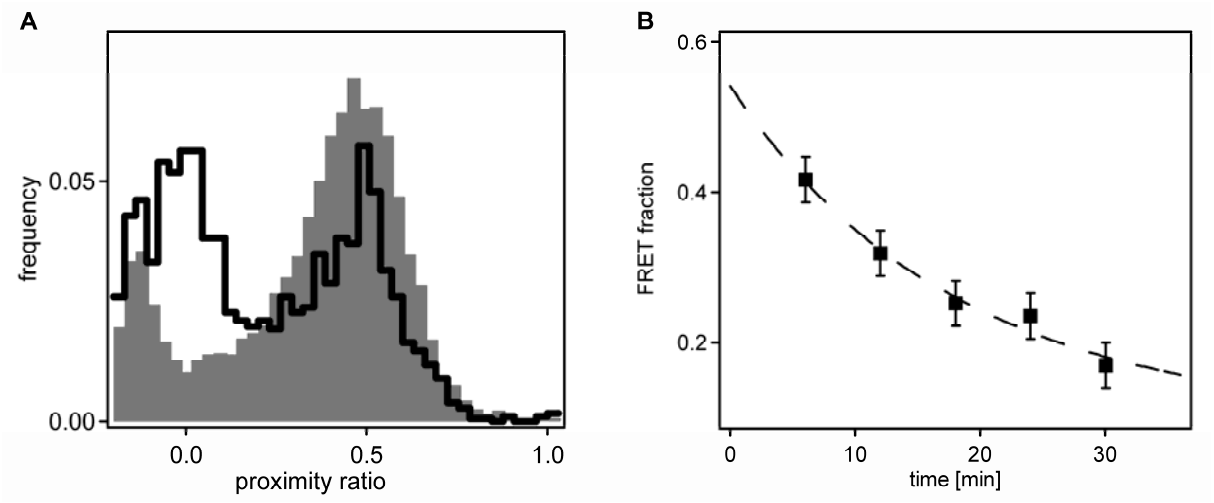
H2A-H2B dimer can exchange between nucleosomes confirming H2A-H2B dissociation
Comparison of FRET histograms from four samples: A) mixture of nucleosomes labeled at H2B (donor) and nucleosomes labeled at the DNA^{-52} (acceptor) at 760 mM NaCl, B) same sample, incubated at 760 mM NaCl and diluted to 380 mM NaCl, C) double labeled nucleosomes H2B-DNA^{-52} at 150 mM NaCl as a reference, D) same sample as in A, but incubated at 380 mM NaCl and diluted maintaining 380 mM NaCl.
The appearance of a FRET population shows that dimer exchange between nucleosomes can be induced by changing [NaCl]. Comparison with double labeled nucleosomes shows that the dimer binds to its original position within the nucleosome.

Figure S4



Diffusion coefficients measured during reconstitution of H2B-, H4- and DNA- labeled nucleosomes (green, black, red respectively) relative to a standard (alexa 488) as a function of [NaCl] (error bars represent standard deviation from 6 measurements). 210 bp DNA fragments were used in order to increase the difference between the diffusion coefficients of free histones and histone-DNA complexes. Reducing [NaCl] initially causes a decrease of the diffusion coefficient of H4- labeled samples, followed by a decrease for H2B-labeled samples, which indicates a sequential binding of $(H3\text{-}H4)_2$ and H2A-H2B to DNA. The increase of the diffusion coefficient of DNA- labeled samples confirms nucleosome formation.

Figure S5



Influence of experimental conditions on the nucleosome stability
(A) Proximity ratio distribution of DNA^{+42}-DNA^{-52} nucleosomes at 860 mM NaCl and 20 nM (grey shaded) or 250 pM (black) nucleosome concentration. While at 20 nM nucleosome concentration 80 % of the nucleosomes displayed FRET, at 250 pM nucleosome concentration only 50 % of nucleosomes displayed FRET (both samples contain 10 % donor-only labeled particles). This shows that the difference in nucleosome concentration in the experimental setups for FRET and FCS could cause that the structural changes occur at different [NaCl].
(B) The course of the FRET fraction of H4-DNA^{-15} nucleosomes over 30 minutes at 1000 mM NaCl and 250 pM nucleosome concentration. The FRET fraction decays exponentially after dilution in buffer containing high [NaCl] indicating that the difference in incubation time between the analysis of nucleosome disassembly and assembly could cause the differences of [NaCl] observed in these FCS and spFRET experiments.

Figure S6

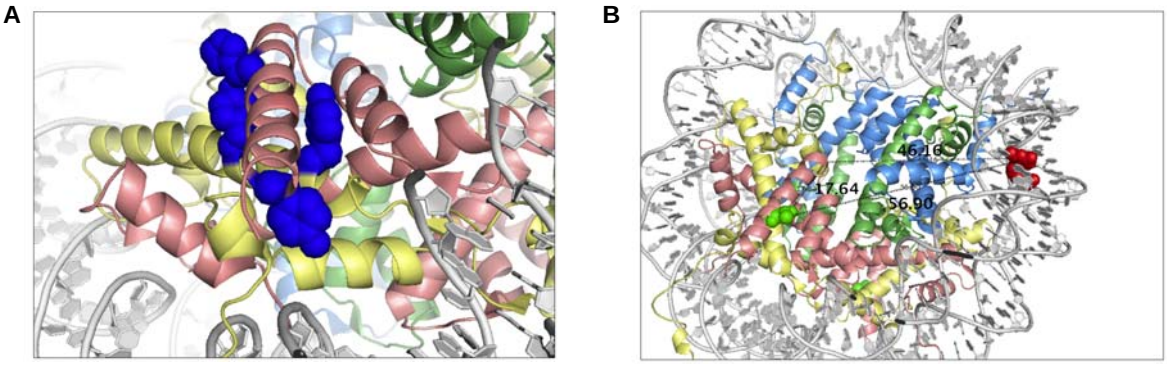


Illustration of the stability of H2B αC helix in its local environment
(A) H2B αC (in red) is firmly held in place by aromatic residues (H2A – Y57, Y50; H2B – H106, Y118 in blue).
(B) Movement of H2BαC does not significantly change the distance of the two fluorophores. The H2B/DNA^{-15} dye-to-dye distance is approximately 57 Å. We calculated a distance of 18 Å between C112 and G101, and 46 Å between G101 and position -15 on the DNA which can be summed for a maximum linear distance of 64 Å.

5. Tables

Table S1. Distances between the positions carrying the donor- and acceptor-fluorophore within the nucleosome, calculated using PyMOL

Construct	Distance [nm]	Distance [nm]
H2B-DNA^{-15}	5.6	5.9
H2B-DNA^{-52}	4.2	6.1
H4-DNA^{-15}	3.2	5.4
H4-DNA^{-52}	4.9	5.8
DNA^{+42}-DNA^{-52}	5.9	-

Table S2. Relative diffusion coefficients of free H2A-H2B and H2B-labeled nucleosome samples at low and high [NaCl] (the standard deviation of 10 measurements is given as the ± value)

Sample / [NaCl]	free dimer	nucleosome sample labeled at H2B
300 mM	0.21±0.01	0.11±0.01
1100 mM	0.23±0.01	0.21±0.01

Table S3. Number of ion pairs involved in the intranucleosomal interactions calculated from the correlation of nucleosome disassembly and [NaCl], as described in section 2.7 (the standard deviation of the fit is given as the ± value).

interaction	construct	# ion pairs
octamer internal	H2B-DNA^{-15}	4±1
histone dimer - DNA	H2B-DNA^{-52}	11±1
histone tetramer- DNA	H4-DNA^{-15}	24±2
	H4-DNA^{-52}	23±2
	DNA^{+42}-DNA^{-52}	20±2