Exploring Nucleosome Unwrapping Using DNA Origami

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Supporting Information

ABSTRACT: We establish a DNA origami based tool for quantifying conformational equilibria of biomolecular assemblies as a function of environmental conditions. As first application, we employed the tool to study the salt-induced disassembly of nucleosome core particles. To extract binding constants and energetic penalties, we integrated nucleosomes in the spectrometer such that unwrapping of the nucleosomal template DNA, leading from bent to more extended states was directly coupled to the conformation of the spectrometer. Nucleosome unwrapping was induced by increasing the ionic strength. The corresponding shifts in conformation equilibrium of the spectrometer were followed by direct conformation imaging using negative staining TEM and by FRET read out after gel electrophoretic separation of conformations. We find nucleosome dissociation constants in the picomolar range at low ionic strength (11 mM MgCl2), in the nanomolar range at intermediate ionic strength (11 mM MgCl2 with 0.5−1 M NaCl) and in the micromolar range at larger ionic strength (11 mM MgCl2 with ≥1.5 M NaCl). Integration of up to four nucleosomes stacked side-by-side, as it might occur within chromatin fibers, did not appear to affect the salt-induced unwrapping of nucleosomes. Presumably, such stacking interactions are already effectively screened at the nucleosome unwrapping conditions. Our spectrometer provides a modular platform with a direct read out to study conformational equilibria for targets from small biomolecules up to large macromolecular assemblies.

KEYWORDS: DNA origami, nucleosome, FRET, TEM, force spectroscopy

Programmable self-assembly with DNA origami allows creating custom-shaped nanoscale objects. Through this capacity, DNA origami enables constructing custom instruments to perform precision measurements of molecular interactions and structure, with enhanced control over positioning, orientating and manipulating the molecules under study. Researchers have just begun to exploit this capacity, for example, to study the collective behavior of molecular motors, to augment stochastic sensing with nanopores, to enhance NMR-based structure measurements, to study vesicle fusion, and to perform measurements of weak intermolecular forces such as basepair stacking or the interactions between nucleosomes. Here, we establish a tool for quantifying conformational equilibria that leverages the control over the placement of molecules on the nanoscale. The system enables coupling and amplifying structural changes of the molecule under study for detection via transmission electron microscopy (TEM) imaging, fluorescence measurements, and gel electrophoresis.

Our system builds on a previously described object consisting of two rigid DNA origami beams connected by a hinge. The object was previously used for controlling the distance between two molecules with subnanometer accuracy. The hinge acts as a torsional spring that biases the configuration of the device toward a subset of preferred opening angles. Through this property, the object may be employed to exert forces on molecular binding partners, and it becomes thus a type of force spectrometer. One may use the device, for example, to quantify the forces between two molecular surfaces by analyzing the change in the distribution

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of opening angles that are adopted by the device with and without the forces between the molecular surfaces under study. Using this concept, we previously determined the distance-dependent energy landscape for the weak stacking interactions between two nucleosome core particles (hereafter just “nucleosomes”). Also, Castro and co-workers recently employed a related device to explore the linker-length dependence of partial unwrapping of nucleosomes.18

Our device should also allow studying conformational equilibria of biomolecular assemblies as a function of environmental conditions. Inter- and intramolecular conformational equilibria may, of course, be studied by various existing methods, in particular using FRET spectroscopy.19−22 However, the DNA origami spectrometer system offers a number of features that promise added value in such measurements: First, the binding partners under study may be displayed in close vicinity, in controlled orientations, and in controlled stoichiometry, which provides opportunities for interrogating multivalent interactions, next-neighbor interactions, and the effect of relative orientations. Second, a force bias may be exerted in a particular way to prepare otherwise rarely populated states. Third, the signals reported by the device (opening angles or FRET efficiencies) are distance-calibrated with near-atomic accuracy.16 Fourth, the dynamic range for molecular distance changes may be adjusted and expanded much beyond the typical range of FRET by placing the dye pair at different positions on the device.15 Fifth, simple gel electrophoresis may be used in a standardized fashion as read out for the conformational equilibrium. Potential difficulties arise from the need to conjugate the molecular targets to the spectrometer, but for conventional FRET studies also the fluorescent dyes would need to be conjugated to the molecule(s) under study.

The goal of the present study is to use the spectrometer for quantifying conformational equilibria and their dependence on environmental conditions which requires establishing the necessary experimental methods and data analysis. To this end we use imaging by TEM to track the conformational equilibrium of a molecular complex with direct structural feedback on the single particle level. On the ensemble-level and complementary to TEM, we monitor the equilibrium using FRET. As a first application, we study the salt-induced disassembly (“unwrapping”) of nucleosomes. The nucleosome is a disc-shaped DNA–protein complex with a diameter of ∼10.5 nm and a height of ∼4.5 nm23−25 in which a 147 base pairs (bp) long DNA double-strand is wrapped 1.65-times around an octamer of histone proteins. Two copies of each histone protein (H2A, H2B, H3, and H4) form the histone octamer. In a nucleosome, the positively charged proteins interact with the negatively charged DNA. Therefore, the stability of this DNA–protein complex strongly depends on the ionic strength of the solution.26,27 In vitro, the nucleosome can be reconstituted from a mixture of template DNA and histone octamers by salt gradient dialysis.28 The assembly proceeds through three major steps:23,29,30 the (H3−H4)2 tetramer associates to the DNA template at ∼1 M NaCl forming the intermediate tetradsome; the H2A−H2B dimers associate to the tetradsome at ∼0.8 M NaCl forming a partly wrapped

Figure 1. Placing nucleosomes on a DNA-origami force spectrometer. (A) Schematic illustration of the force spectrometer with two attached nucleosomes in a configuration indicated by the icon in the bottom left. Solid elements indicate occupied nucleosome sites. (B) Schematic illustration of the force spectrometer without (left) and with (right) attached nucleosomes. The flexible hinge of the spectrometer allows for a continuum of opening angles (left), while the angle is constrained upon nucleosome attachment. (C) Schematic illustrations of spectrometer top view, average electron micrographs of spectrometer top- and side view for four out of 16 possible nucleosome configurations. Icons indicate nucleosome configurations as in A. White arrows indicate nucleosome positions. Scale bar: 25 nm. (D) Magnified average electron micrographs from C highlighting attached nucleosomes. Brightness levels were adjusted across micrographs such that the brightness of the spectrometer beams was set equal. Scale bar: 10 nm. NaCl was added to a final concentration of 500 mM immediately prior to the TEM grid preparation for images shown in C and D to increase adsorption to the TEM grid surface.
nucleosome that relaxes at 0.6 M NaCl to a completely wrapped state. The salt-induced disassembly follows the pathway in reverse order.

We integrated the nucleosomes in the spectrometer so that a 41 bp-long DNA segment remains in the spectrometer connecting both beams (Figure 1, Figure S1). In the wrapped versus unwrapped state of the nucleosome, the template segment will be bent versus extended, respectively, which in turn will cause the spectrometer to adopt a smaller versus larger opening angle (Figure 2A). The opening angle thus becomes an

Figure 2. Salt-induced nucleosome disassembly studied with electron microscopy. (A) Left: Schematic representation of the force spectrometer with an attached nucleosome and with a FRET pair (D = ATTO550, A = ATTO647N) mounted 5.4 nm away from the hinge. Right: Force spectrometer after salt induced release of the histone octamer (blue), a 41 bp-long DNA segment remains in the spectrometer connecting both beams. (B) Negative staining electron microscopy field of views of spectrometers that where incubated with nucleosomes at 11 mM MgCl₂ without (left) and with (right) 1.0 M NaCl. Green circles indicate spectrometers with attached nucleosomes. Icon (top left) indicates nucleosome configuration as in Figure 1. Scale bar: 50 nm. (C) Uniform kernel density estimates (bandwidth 3°) for the opening-angle probability densities of spectrometers with one binding site (as in B) to which nucleosomes were attached and indicated amounts of NaCl were added. Particles were classified manually into classes with (green) or without (red) nucleosome density at the designed location. (D) Exemplary reference free negative staining electron microscopy class averages and magnified regions as in Figure 1D but for the samples of experiments described in C. Circles indicate expected nucleosome dimensions (diameter of 10.5 nm). Scale bars: 10 nm.
indicator for the conformation of the nucleosome, specifically, for its degree of unwrapping. In addition, we configured the spectrometer so that it may host up to four nucleosomes in an arrangement which may be likened to a stack of tires (Figure 1A). This arrangement allows determining penalties for integration and enables also testing whether nucleosome stacking interactions affect unwrapping transitions. For the coupling, we used nucleosomes based on the Widom 601 sequence\(^3\) that bear DNA single-strands as previously described\(^1\) (Figure S2). The nucleosomes were reconstituted by salt gradient dialysis with a branched DNA template such that two DNA single strands protruded radially and opposite from each other from the nucleosome. We hybridized those strands to complementary single strands that are displayed at selected positions on the two beams of the spectrometer (Figure 1A,B and Figures S1 and S3).

**Results and Discussion.** We prepared spectrometer variants featuring one, two, or four nucleosomes (Figure 1C) and imaged these samples using negative-staining transmission electron microscopy (TEM). The micrographs showed clearly disc-shaped features at the designed positions and in the designed orientations (Figure 1C,D). Moreover, the transmission contrast of the disc features also depended clearly on the number of nucleosomes hosted by the spectrometer (Figure 1D). For example, the disc features in the samples designed to include one versus four nucleosomes had the lowest versus highest transmission contrast (Figure 1D). Thus, TEM imaging confirmed the successful incorporation of nucleosomes into the spectrometer according to design.

To reveal the salt-induced disassembly of nucleosomes (Figure 2A) by TEM, we imaged spectrometer particles containing one nucleosome (same sample as in Figure 1C) in the presence of four different concentrations of sodium chloride (see Figures S4 and S5 for representative micrographs). As the nucleosomes could be clearly discerned in individual particle micrographs, we could sort the image data according to whether or not the particles featured a nucleosome. We measured the opening angles of individual spectrometer particles and determined the frequency at which particular angles were populated in the particle subsets with and without nucleosome. The frequency of particles featuring nucleosomes clearly decreased with increasing salt concentration (Figure 2B,C). In the absence of sodium chloride, 52% of the particles had integrated nucleosomes, and the distribution of opening angles for those particles peaked at 50° opening angle. The particles lacking a nucleosome sampled a broader spectrum of opening angles that was shifted toward greater opening angles (Figure 2C). The nucleosome-free distribution was consistent with previous measurements\(^1\) and reflects the conformational freedom of the spectrometer without an element connecting the two beams. In the presence of 500 mM sodium chloride, the fraction of particles with nucleosomes remained approximately constant at 58%, but the angle distribution for particles with nucleosomes became narrower compared to the one obtained in the absence of sodium chloride (Figure 2C). Small opening angles below 40° were more rarely populated in the presence of 500 mM NaCl than in the absence of NaCl. Close inspection of the particles having small opening angles <40° at zero NaCl indicated elliptical rather than the circular disc features of the particles with larger opening angles (Figure S6). The elliptical features may indicate a tilted orientation of the nucleosome. Possibly, enhanced interactions with the charged TEM support surface at elevated ionic strengths suppress the tilted orientation.

Upon increasing the sodium chloride concentration further to 1 M and then to 1.5 M, the fraction of particles featuring nucleosomes decreased drastically to 32% and 5%, respectively (Figure 2C). At the same time, a new peak at 70° emerged in the angle distribution for the particles without nucleosome. We attribute this population to particles where the histones dissociated from the template DNA, which is then free to relax from a bent to an extended conformation. In this relaxed state, a 41 bp-long double-helical segment of DNA bridges the two beams of the spectrometer. According to our geometric model of the hinged-beam device,\(^1\) such a 41 bp DNA bridge in a straight configuration should lead to an average opening angle of 67° ± 2°, which corresponds very well to the observation. In addition to manually analyzing particles, we also performed reference-free classification and averaging of particles for the four different salt concentrations. The nucleosome density in the average micrographs fades away with increasing salt concentration, reflecting the decrease in frequency of particles with a nucleosome. Inspection of the various angle classes at different salt concentrations did not reveal signs of partially unwrapped nucleosomes within the resolution of the image data; whenever nucleosome density could be discerned, it was disc-shaped, which supports an apparent two-state disassembly transition (Figure S6).

The TEM data confirm that the spectrometer conformation is closely tied to the presence of intact nucleosomes and clearly reflect salt-induced disassembly of nucleosomes consistent with previous observations.\(^7\)\(^9\)\(^32\) Hence, reading out the state of the spectrometer can report on the state of the incorporated DNA–protein complex. We now exploit this property to quantify the nucleosome unwrapping transition with more widely available equipment, i.e., gel electrophoresis combined with fluorescence imaging. To this end we integrated a FRET dye pair in the beams of the spectrometer to provide a fluorescent signal that reports on the opening angle of the spectrometer in solution\(^1\) (Figure 2A).

Our goal is to use this setup, in conjunction with a model, to quantify the free energy difference between wrapped and unwrapped states, with the histones bound on the template DNA in the spectrometer versus free in solution. However, as the hinge corresponds to a torsional spring the bound state is destabilized in our system by the energetic penalty for closing the spectrometer. On the single particle level, the bias may be calibrated by collecting angle distributions as previously described.\(^1\) In the context of bulk FRET measurements, integrating more than one nucleosome in the spectrometer provides an alternative route for determining the contributions from closing the spectrometer and unwrapping a nucleosome. We prepared 15 versions of the spectrometer that sample all possible combinations for integrating at least one and up to four nucleosomes at the four available positions in the spectrometer (see Figure 1). The samples were incubated in buffers containing various sodium chloride concentrations, and the resulting conformations were separated by agarose gel electrophoresis. The equilibrium distribution was preserved during electrophoresis as the low-salt electrophoresis buffer stabilized existing nucleosomes and prevented, together with dilution of the samples during electrophoretic separation, de novo nucleosome formation. We produced a set of three images per gel using a fluorescence laser scanner: the donor emission intensity upon donor excitation, the acceptor emission emission
intensity upon donor excitation, and the acceptor emission intensity upon acceptor excitation. By computing the FRET efficiency individually for each image pixel (Supporting Note 1) we obtained images that simultaneously report on the electrophoretic mobility and the FRET efficiency signal per band (Figure 3A,B). Based on the TEM experiments described in Figure 2, we can expect three different species: particles with integrated nucleosomes, particles with dissociated histone proteins but template DNA bridging the spectrometer beams, and particles lacking nucleosomes and template DNA. Particles with intact nucleosomes gave a high FRET signal (around 0.6). Due to larger spectrometer angles in the presence of the extended versus the bent template DNA segment bridging the spectrometer beams, particles with disassembled nucleosomes had low FRET efficiency (around 0.4). Particles that lacked the template in the first place also had low FRET efficiency (around 0.36).

We performed two different types of experiments: in one type we varied the sodium chloride concentration in a wide range from 0 to 2.5 M for three different nucleosome-loaded spectrometer variants (with one, two, and four nucleosomes) (Figure 3A). The samples with a particular spectrometer variant but different salt conditions were electrophoresed on the same gel in order to directly compare absolute electrophoretic mobility and FRET signals. In the second type of experiment, we electrophoresed the full set of 15 different nucleosome-loaded spectrometer variants together on one gel after incubation at identical salt concentrations (Figure 3B). This experiment was repeated for three different salt concentrations in the regime where the unwrapping equilibrium is sensitive (i.e., between 0.9 and 1.1 M NaCl).

The gel image data revealed that spectrometer particles designed to host only one nucleosome and incubated at low salt concentrations migrated in two overlapping leading bands (Figure 3A). The faster band had a high FRET efficiency, while the slower band showed a low FRET efficiency. We attribute the faster, high-FRET band to spectrometers with correctly attached and intact nucleosome particles. The slower low FRET band should reflect particles lacking nucleosomes with template strand attached to both arms. In samples incubated with higher sodium chloride concentrations (around 0.5 M NaCl), the fast, high-FRET band disappeared, indicating that nucleosome disassembly occurred. The appearance of the slower low FRET band also changes: it becomes broader and shifts upward, which we attribute to the emergence of particles with dissociated proteins and remaining template segment that bridges the two spectrometer beams. In the sodium chloride titration series obtained with two or four nucleosomes, the unwrapping transition is completed at 0.9−1.1 M NaCl. We note that at higher ionic strengths also the extent of spectrometer aggregates increases; i.e., more material remained in or close to the gel wells. We speculate that the disassociating

Figure 3. Salt-induced nucleosome disassembly studied with gel electrophoresis and ensemble FRET. (A, B) Images of gel electrophoresed force spectrometers with nucleosomes attached in configurations indicated by the icons as in Figure 1 (solid elements = site is occupied with a nucleosome), which were incubated at the indicated ionic strength prior to gel electrophoresis. The color of each pixel indicates the FRET efficiencies calculated from laser-scanned gel images (see Supporting Note 1 for detailed image analysis). P = pocket, D = spectrometer dimer, c = closed spectrometer, o = open spectrometer. (A) Exemplary experiments of spectrometers with the same nucleosome configuration, but different amounts of additional NaCl. Samples were electrophoresed for ~7 h in 3% agarose gels to separate closed and open spectrometers. (B) Exemplary experiments of spectrometers with different nucleosome configurations, but same amounts of additional NaCl. Samples were electrophoresed for 2.5 h in 2% agarose gels. (C) Closed fraction of spectrometers determined from the gel electrophoresis. Circles: data from experiments as shown in A; solid lines: fits to a noncooperative sequential binding model (Supporting Notes 2 and 3 for data analysis and fit of model). (D) Parameters of the model from the fit shown in C. (E) Dissociation constants computed from the parameters in D for different salt concentrations.
histone proteins promote this aggregation. At low ionic strength, for the samples designed to host two or four nucleosomes we observe a single fast band with high FRET efficiency. This observation indicates that in these samples each spectrometer particle hosts at least one wrapped nucleosome with the template DNA bridging the two spectrometer arms (see, e.g., Figure 3A bottom, lane 1). For the second type of experiments with the 15 spectrometer variants, a trend is clearly visible, in which the leading bands in samples having more nucleosomes tend to have greater FRET efficiencies at a given salt condition (Figure 3B). The greater FRET efficiency signals indicate that a greater fraction of particles host intact nucleosomes at a given condition.

For the analysis of this data, we computed the average FRET efficiency per band (Supporting Note 1) and corrected the FRET efficiency for finite nucleosome attachment yield (which depended on position in the spectrometer, Supporting Note 2). This accounts for spectrometer particles lacking a template DNA. The FRET values can be associated with the fraction of closed spectrometer particles as caused by the presence of at least one wrapped nucleosome on a bridging template per spectrometer (Supporting Note 2). As a result, we obtain a graph of the closed fraction as a function of sodium chloride concentration along one axis and as a function of the number of nucleosomes in the system along the other axis (Figure 3C, S8).

These FRET data from the two types of gel experiments provide sufficient constraints to estimate energetic parameters for the nucleosome unwrapping equilibrium using a model for the reaction. A first simplified equilibrium reaction model for nucleosome disassembly (Supporting Note 3) considers five species: free histone octamers, free template DNA, spectrometer with extended template DNA and dissociated histones, spectrometers with bent template DNA and i intact nucleosomes on n possible binding sites (subsumed as one species), and free nucleosomes formed between free template DNA and histone octamer. The model strongly simplifies nucleosome formation as a bimolecular reaction between the histone octamer and template DNA. For the reaction between free templates and histone octamers, we assign a free energy difference $\Delta G_{\text{wrap}}$ that depends linearly on the sodium chloride concentration. $\Delta G_{\text{wrap}}$ is also taken for forming a nucleosome in the context of the spectrometer; however, for the reaction between spectrometers with zero nucleosomes already bound and a histone octamer, we assume an additional closing penalty of $\Delta G_o$. The binding and unbinding of individual histone octamers is assumed to occur independently from the state of other neighboring nucleosomes in the spectrometer. All in all, the model has only three free parameters, i.e., the closing penalty $\Delta G_o$, the free-energy difference $\Delta G_{\text{wrap}}$ at zero sodium chloride concentration, and the free-energy increment per change in sodium concentration. With this model we could describe our FRET data set successfully within the margin of error. A global fit yields a free energy difference for nucleosome unwrapping of $-14.5 \text{ kcal/mol}$ at zero sodium, which corresponds to very tight binding with a dissociation constant of around 50 pM for the nucleosome unwrapping equilibrium (see Figure 3D,E). At 500 mM sodium chloride, the equilibrium shifts to the $\sim1$ nM dissociation constant regime which is consistent with the range of previously determined values at physiological conditions (i.e., from 0.03 to 3 nM$^{-1}$). At 1.5 M sodium chloride, we have weak binding in the micromolar regime. The value for the energetic penalty derived from the global fit (1.2 kcal/mol) is consistent within margin of error to the penalties for closing to angles between 30° and 50° (i.e., 1.2–0.5 kcal/mol) that were determined previously and independently by TEM imaging.15

The nucleosomes were sufficiently close to each other in our spectrometer so that stacking interactions may take place between them.15 On the other hand, their proximity may also cause an entropic penalty because a rotational degree of freedom will be suppressed. We therefore also considered a second model that includes interactions between neighboring nucleosomes in the context of the spectrometer (Supporting Note 4 and Figure S9). However, this model did not result in an improved description of the data within the margin of error. Presumably, such stacking interactions are already effectively screened at the nucleosome unwrapping conditions.

**Conclusion.** This study establishes that the DNA origami force spectrometer may be employed to quantify conformational equilibria as a function of environmental conditions. As readout, the user may rely on direct imaging of particle populations via TEM, or the user may alternatively use ensemble-level fluorescence signals and gel electrophoresis. The system further allows for integrating multiple copies of the complex under study in particular orientations, which allows us to investigate systems where multivalent interactions, next-neighbour interactions, and relative orientations may play a role. The spectrometer’s range of sensitivity can be adapted to the distance changes in the molecular system under study by moving the dye-pair positions and the molecular attachment sites along the spectrometer beams. This extends the range of FRET, which is typically limited to distances of 1–10 nm.36 Indeed, our force spectrometer enabled us to monitor nucleosome unwrapping along an intramolecular coordinate that changed from 10 to 15 nm upon nucleosome unwrapping. Because of the modularity and addressability of the DNA origami based device, many other biomolecular complexes may be studied with the spectrometer. For example, quantitative protein folding studies in equilibrium are conceivable in which our spectrometer tracks the separation between two sites on the protein surface. Such experiments should be possible due to the high structural stability of DNA origami objects in the presence of chaotropic agents.36 In conclusion, our spectrometer provides a modular, versatile, and adjustable platform with a direct read out to study conformational equilibria for targets from small biomolecules up to large macromolecular assemblies.

**Materials and Methods.** The design, assembly, and purification of the DNA origami force spectrometer were performed as described in refs 15 and 37–39. Four orthogonal sets of adapter sequences were designed with NUPACK40 and protrude from positions indicated in the design diagram (Figures S1, S2, and S3).

The reconstitution of nucleosomes with single-stranded DNA handles was performed as described in refs 15 and 28 with purified histone octamers from *Drosophila melanogaster* embryos and branched DNA templates based on the 601 sequence31 (Figure S2).

The attachment of nucleosomes to the force spectrometers was done as described in ref 15. Briefly, 40 nM of spectrometers featuring single-stranded DNA attachment handles at designed attachment sites were incubated with nucleosomes in buffer containing 11 mM MgCl$_2$, 5 mM Tris base, 1 mM EDTA, and 35 mM NaCl (pH $\sim$ 8) at 4 °C overnight. 135 nM of nucleosomes for each binding site on the spectrometer with the corresponding set of complementary sequences were used.
Negative staining TEM grid preparation and imaging analysis of spectrometers with attached nucleosomes was performed as described in refs 15 and 41.

Salt-titration experiments were performed in 11 mM MgCl₂, 5 mM Tris base, 1 mM EDTA, and 35 mM NaCl (pH ~ 8) for 1–2 days at room temperature (∼25 °C) with the indicated amounts of additional sodium chloride. We confirmed that our systems are equilibrated after a 1 day incubation with NaCl at room temperature by comparing salt-titration experiments that were incubated for 1 and 3 days (Figure S10).

Gel electrophoresis of nucleosome-loaded force spectrometers with different nucleosome configurations (Figure 3B) at constant NaCl concentration was performed in 2% agarose gels for 2.5 h at 70 V. Gel electrophoresis of nucleosome-loaded force spectrometers with the same nucleosome configuration but varying NaCl concentrations (Figure 3A) was performed in 3% agarose gels for 7.5 h at 70 V. In all gel electrophoretic experiments, both the agarose gel and the running buffer contained 11 mM MgCl₂ and 0.5X TBE (1 mM EDTA, 44.5 mM Tris base, 44.5 mM boric acid, pH ~ 8.3). Gels were imaged with a Typhoon Flx 9500 (GE Healthcare) and analyzed with a custom-built MATLAB software (The MathWorks, Inc., Natick, Massachusetts, United States) (Supporting Note 1).

■ ASSOCIATED CONTENT

Supporting Information

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Supporting figures and notes (PDF)

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J.J.F., P. Ketterer, and C.L. performed the research. H.D. designed research. J.J.F. and P. Ketterer prepared spectrometers and collected and analyzed TEM and FRET data. C.L. prepared and characterized nucleosomes under the supervision of P. Korber. J.J.F. and H.D. wrote the manuscript. All authors commented on the manuscript. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supporting Information. Additional data related to this paper may be requested from the authors.

Notes

The authors declare no competing financial interest.

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■ REFERENCES


