Geometry Based Self-Assembly of Histone-DNA Nanostructures at Single-nucleotide Resolution

Maged F. Serag,^{1,2,3} Aimaity Aikeremu,¹ Ryoko Tsukamoto,^{2,3} Hubert Piwoński,¹ Maram Abadi,¹ Noritada Kaji,⁴ Jason R. Dwyer,⁵ Yoshinobu Baba,^{2,3,6,7} Satoshi Habuchi^{1*}*

¹ Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

² Department of Biomolecular Engineering, Graduate School of Engineering, Nagoya University.

³ ImPACT Research Center for Advanced Nanobiodevices, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

⁴ Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 819-0395, Ja-pan

⁵ Department of Chemistry, University of Rhode Island, 140 Flagg Road, Kingston, RI, 02881, USA

⁶ National Institute of Advanced Industrial Science and Technology (AIST), Takamatsu, Japan.

⁷ School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

*Correspondence should be addressed to Maged.Serag@kaust.edu.sa and Satoshi.Habuchi@kaust.edu.sa.

Abstract

Histones are basic protein monomers capable of interacting with DNA, providing the mechanism of DNA compaction inside the cell nucleus. The well-ordered assembly process of histone and DNA is a potential candidate as the approach for building DNA-protein nanostructures. Here, utilizing the sequence-independent histone-DNA interaction, we present an approach to self-assemble histones and single-stranded DNA (ssDNA) to form well-defined histone-DNA (sHD) nanoparticles and their multidimensional cross-linked complexes (cHD). By using various molecular biology and microscopy techniques, we elucidate the structure of these complexes, and we show that they are formed at carefully controlled conditions of temperature, ionic-strength, concentration and incubation time. We also demonstrate using a set of ssDNA molecular rulers and a geometric accommodation model that the assembly of sHD and cHD particles proceed with precise geometry so that the number of ssDNA in these particles can be programmed by the length of ssDNA. We further show that the formation of cHD amplifies the effect of the length of ssDNA on the self-assembly, allowing for distinguishing ssDNA of different lengths at single nucleotide resolution. We envision that our geometry-directed approach of self-assembling histone-DNA nanostructures and the fundamental insights can serve as a structural platform to advance building precisely-ordered DNA-protein nanostructures.

Keywords

single-stranded DNA, histone, self-assembly, DNA-protein hybrid, nanotechnology

Integrating proteins into DNA-based nanostructures can create a promising platform in the field of DNA and protein nanotechnology.^{1,2} This platform promises important applications in medicine,³ biosensing,⁴ biomolecular analysis⁵ and biomimetics.⁶ Notably, the self-assembly of proteins and DNA to form higher-order protein-DNA hybrid nanostructures has been realized and conceived to impact fundamental research and industrial biotechnology.¹ Indeed, the availability of proteins that are capable of self-assembly with DNA is a fundamental prerequisite to leverage this technology and to broaden its applications. Histones, a family of proteins termed H1, H2A, H2B, H3 and H4, are potential candidate of proteins for the hybrid self-assembly because they have intrinsic property to associate with DNA to form nucleosomes.

The mechanism of nucleosome formation involves the dsDNA-promoted assembly of a core particle consisting of two copies of four histone monomers (H2A, H2B, H3 and H4) to form a dsDNA-wrapped histone octamer. Nucleosomes are interconnected by segments of dsDNA that bind to the fifth histone monomer (H1).⁷ Nucleosome formation is a highly ordered process that requires the sequential binding of an (H3–H4)² tetramer followed by binding to two H2A–H2B dimers.⁸ This ordered assembly can be achieved *in vitro* by salt gradient dialysis that relies on the fact that (H3–H4)₂ tetramers binds to DNA at higher salt concentration compared with H2A–H2B dimers.⁹ The assembly can be also achieved *in vivo* and *in vitro* by chaperone guidance.¹⁰ Chaperones are proteins that form histone-chaperone complexes and promote the ordered addition of histone subunits along dsDNA. Histone octamers interact with dsDNA through 14 spirally-arranged docking sites. The distance between these sites, along the spiral pathway is approximately 4 nm (Figure 1). Single-stranded DNA (ssDNA) similarly stabilizes histone octamers (ssOct) *via* the stepwise management of the solution ionic strengths^{11,12} (Figure 1a). The wrapping of ssDNA oligomers around histone octamers always follows the best way of neutralization of the negatively

charged phosphate groups.^{14,15} It has been posited that two ssDNA oligomers can bind to the 14 docking sites and stabilize histone-octamers through a spiral alignment similar to nucleosome. One model proposed that two ssDNA molecules could line up side by side (without being base-paired) around the histone octamer (denoted double-copy wrapping).¹² Another model proposed that at least 2 molecules of ssDNA could bind to the histone-octamer, such that each ssDNA molecule interacts with one heterotypic tetramer.¹² Several studies have shown that short pieces of ssDNA could also reconstitute histone octamers having similar core structure, morphological characteristics, hydrodynamic behavior and the same DNA mass as their analogues stabilized with longer dsDNA.¹¹⁻¹⁷ These ssDNA wrapped octamers tend to cross-link in solution *via* the dangled ssDNA tails to form a network of cross-linked histone octamers (CLO). These complexes are soluble in low ionic strength buffers (*e.g.* 1mM Tris buffer) and precipitate in higher ionic strength buffers.¹²

Taken together, the ordered assembly of nucleosome and its ssDNA-wrapped analogue (ssOct) is mediated either by chaperones or by gradient dialysis.¹⁸ This guided-assembly is complicated and thus hampers the utilization of these complexes and their assembly approaches to build structurally organized DNA-protein nanostructures. Therefore, we hypothesized that if the inherent ability of histone to associate with ssDNA can be utilized to self-assemble a fundamental building-block unit, this self-assembly would be an essential step to construct pre-designed DNA-protein hybrid nanostructures. To that end, we devised an experimental method that enables the self-assembly of well-defined histone-ssDNA nanostructures. In addition, by means of various microscopy techniques, qualitative and quantitative gel electrophoresis, ssDNA molecular rulers, fluorescence photon coincidence measurement, electrophoretic single particle tracking and a geometric accommodation model, we characterize the multiscale mechanism of histone-ssDNA

interactions from nucleotide-scale to subunit-scale level, and describe how to tune the stability of the histone-DNA complexes. Knowledge of this stability and the underlying mechanism is essential for a bottom-up design approach of stable, organized protein-DNA hybrid nanostructures.

RESULTS AND DISCUSSION

Experimental design

To explore the possibility of *in vitro* self-assembly of histone monomers by ssDNA, we used polythymine ssDNA, human recombinant histone monomers (H2A, H2B, H3 and H4; RecH), histone subunits extracted from calf thymus (H1, H2A, H2B, H3 and H4; collectively denoted CTH; Figure S1 and S2), and H1-depleted CTH (CTH^{H1}, Figure S1). We used ssDNA instead of dsDNA because its high structural flexibility could increase the stability of histone-ssDNA intermediates¹⁹ and because the sticky ends that dangle from an assembled structure¹² could be used to connect to a DNA origami motif. We used polythymine ssDNA sequences to avoid the formation of secondary structures arising from intra-chain self-stacking. It is well known that repetitive DNA sequences fold into non B-DNA structures such as hairpin, cruciform, triplex, tetraplex...*etc.* For example, Polyguanine can fold into quadruplexes, polycytosine can fold into i-tetraplexes and polyadenine can fold into A-motif. The self-stacking interactions in these three oligomers are much stronger than the self-stacking interactions in polythymine.¹³ We used native histone subunits extracted from calf thymus to investigate whether post-translational modification has a useful role to promote the self-assembly, whereas, we used recombinant histone subunits as a negative control. On the other hand, we used H1-depleted CTH to investigate the role of H1 in the self-assembly and the cross-linking processes.

It is well established that the direct interaction of histone with dsDNA at low ionic strength leads to non-specific aggregation.²⁰ Therefore, gradient dialysis is the standard procedure to properly assemble histone octamers wrapped by dsDNA.⁹ To the best of our knowledge, the experiments reported in the literature for nucleosome assembly *via* gradient dialysis were done by using at least 30 μ M CTH. On the other hand, attempts for self-assembly of histone octamers wrapped by short ssDNA oligomers were done by using 10 μ M CTH.¹² Because concentration, ionic strength and temperature play a key role in histone-histone interaction/aggregation (see Note S1) and in the agglomeration and the subsequent precipitation of ssOct and CIO,¹² we investigated whether an exceedingly low ionic strength, concentration and temperature could favor the self-assembly of well-defined histone-ssDNA complexes. Therefore, we studied the interaction of CTH and ssDNA in solution at a low concentration (typically 120 nM ssDNA and 240 nM CTH) and at low ionic strength (1 mM Tris buffer) regime. These concentration and ionic strength regimes are orders of magnitude smaller than those typically used for nucleosome and ssOct assembly, which could promote direct histone-ssDNA interactions beyond those induced by crowding or ionic strength.

Having investigated the interaction of CTH with ssDNA and elucidated the structure of the generated assemblies, we explored the molecular mechanism of this interaction by means of ssDNA molecular rulers. We used ssDNA with 6- to 150-nucleotides (nt) length (6mer-ssDNA – 150mer-ssDNA, end-to-end length of 4.2 – 105 nm). These lengths were selected based on the geometry of histone monomers and octamers; the 4-nm spacing between two binding sites on one monomer and the ~10-nm diameter of the octamer⁷ (the geometric accommodation model, see Figure 1b). The 4.2-nm contour length of the 6mer-ssDNA is barely sufficient to span two consecutive binding sites on one monomer. The 10.5-nm contour length of the 15mer-ssDNA spans three contact sites and therefore could span two monomers *e.g.* H2B and H4 (Figure 1b).

The 35-nm contour length of the 50mer-ssDNA spans 9 binding sites across 5 monomers and therefore could span two heterotypic tetramers. The 45.5-nm contour length of the 65mer-ssDNA spans 12 binding sites and therefore could span 7 (SHL: -6.5 to 4.5) or 8 histone monomers (SHL: -5.5 to 5.5, Figure 1b).

Figure. 1. Experimental design for investigating self-assembly of histone proteins with single-stranded DNA (ssDNA). a) Schematic illustration showing the assembly of histone dimers and ssDNA to form histone octamer (ssOct) and cross-linked octamers (CIO). The diameter (d) of an octamer is approximately 10 nm whereas the DNA pitch (P) is 2.8 nm. DNA wraps 1.67 turns around a histone octamer. b) A schematic illustrating our geometry accommodation model: DNA attaches to 14 binding sites along the spiral pathway around the histone-octamer. The 14 binding sites (yellow circles) can be referenced by their dsDNA superhelical locations (SHL) from -6.5 to

6.5.¹⁸ The geometries of the contour lengths of polythymine (T) ssDNA are shown with respect to the SHL to demonstrate our hypothesized geometric approach not the actual binding of DNA to the SHL.

Optimization of the assembly conditions of CTH by ssDNA

To investigate structural changes induced by the interaction of recombinant and native histones with ssDNA, we tracked changes in the distribution of hydrodynamic particle diameter by quasielastic light scattering (QELS).

As expected, the interaction of CTH and ssDNA in 1mM Tris buffer and at CTH concentration of 10 μ M generated aggregates with a large polydispersity index (PDI = 1, Figure S3). Therefore, we tracked the interaction between CTH and ssDNA at four orders of magnitude smaller than this concentration regime. Figure 2a shows the distribution of measured hydrodynamic diameters (dh) of different histone constructs and different histone-50mer-ssDNA complexes at CTH concentration of 240 nM. We used 50mer-ssDNA because this ssDNA and longer strands consistently generated particles of lower polydispersity index compared with those generated by shorter ssDNA (see below). The self-assembly of 50mer-ssDNA with either RecH, CTH or CTH^{H1} was initially investigated *via* simple mixing at 4 °C and at histone:50mer ssDNA ratio of 2:1. The interaction of RecH with 50mer-ssDNA generated large aggregates with high polydispersity index (PDI = 0.9). In contrast, the interaction of 50mer-ssDNA with either CTH or CTH^{H1} generated particles with three times smaller PDI (dh = 177 $\pm$ 18 nm, PDI = 0.3, Figure 2a). We refer to these low-PDI histone-DNA complexes as HD particles. To further optimize the assembly conditions that result in the formation of HD particles, we studied the effect of temperature (T), incubation time (t), and total concentration (C) of ssDNA and CTH. Because we found no

difference in the generated HD particles by using either CTH or CTH^{H1} and because histone depletion and the successive dialysis steps significantly reduced histone concentration (see Methods and Figure S4), we used CTH instead of CTH^{H1} in this optimization. We found that the HD particles ($dh = 177 \pm 18$ nm) generated at ratio of 2:1 are characterized by smaller polydispersity index (PDI = 0.3) compared with particles generated at lower (PDI = 0.6 at ratio of 0.2) or higher (PDI = 0.7 at ratio of 4) histone:50mer-ssDNA ratios (Figure 2b). RecH, however, generated highly polydisperse particles at different mixing ratios (Figure S5 and Figure S6). Mixing CTH and 50mer-ssDNA at histone: ssDNA ratio of 2:1 and at 4 °C resulted in the formation of HD particles having PDI = 0.17 at $t = 12$ min (Figure 2c), whereas mixing CTH and ssDNA at 25 °C resulted in HD particles with PDI = 0.28 at $t = 6$ min (Figure S7). Furthermore, we found that mixing different concentrations of CTH (0.1 nM ~ 24 μ M) with 50mer-ssDNA (0.05 nM ~ 12 μ M) at histone:ssDNA ratio of 2:1 and at 4 °C generated particles with PDI = 0.17 at 120 nM ssDNA and 240 nM CTH (Figure 2d). Lower and higher concentrations of the ssDNA and CTH at the same histone:ssDNA ratio, however, generated more polydisperse particles (PDI = 0.36 – 1) (Figure 2d). Thus, our results demonstrate that the HD particles generated with 50mer-ssDNA are most efficiently formed (with small PDI) in 1mM Tris buffer at $T = 4$ °C, $C_{ssDNA} = 120$ nM, $C_{CTH} = 240$ nM, $t = 12$ min. We refer to the HD particles that form at these concentrations as HD^x.

Figure. 2. Characterization of the hydrodynamic size of histone-ssDNA assemblies. a) Hydrodynamic diameter of 50mer-ssDNA, recombinant histone monomers (H1, H2A, H2B, H3, H4), calf thymus histone (CTH), H1-depleted CTH (CTH^{H1}) and 50-mer ssDNA:histone complexes. b) Hydrodynamic diameter of histone-ssDNA complexes as a function of histone:50mer-ssDNA ratio ($C_{ssDNA} = 120$ nM, $C_{CTH} = 24 - 600$ nM). The dashed rectangle indicates the range of the mixing ratio at which particles with a polydispersity index (PDI) smaller than 0.3 and hydrodynamic diameter of $d = 177 \pm 18$ nm (denoted by HD particles) were formed. c) Temporal profile of hydrodynamic diameter of histone-ssDNA complexes at histone:50mer-

ssDNA ratio 2 and incubation temperature of 4 °C. The dashed circles indicate the time range at which the HD particles with least polydispersity index were formed. d) Hydrodynamic diameter of histone-ssDNA complexes formed at histone:50mer-ssDNA ratio of 2 and incubation temperature of 4 °C as a function of ssDNA concentration. The dashed square indicates the concentration range at which the HD particles with least polydispersity index were formed. e) Volume distribution quasielastic light scattering (QELS) histograms illustrating the volume vs the hydrodynamic diameter distribution of CTH interacted with 50mer-ssDNA (blue, $C_{\text{ssDNA}} = 120$ nM, $C_{\text{CTH}} = 240$ nM, $T = 4$ °C, $t = 12$ min) and nucleosome (cyan). Right inset: native PAGE of nucleosome. Left inset: native PAGE of HD particles. The arrows show directions of the electrophoresis. The red arrowhead indicates HD particles that were unable to enter the gel, whereas the green arrowhead indicates H1 (see Figure S9 and Note S3). f) QELS histogram illustrating the volume vs. the hydrodynamic diameter distribution of the reconstitutes of CTH and 50mer-ssDNA recovered after the gradient dialysis. HD (dialysis) denotes particles generated by gradient dialysis that lay in the same size regime like that of nucleosome and HD^x particles.

Characterization of the assembled CTH-ssDNA nanostructures

To obtain detailed structural information on the HD^x particles that show small PDI, we characterized their physical size and composition. We first compared the hydrodynamic diameter of the self-assembled HD^x particles with that of nucleosomes that were pre-assembled from recombinant histone monomers and dsDNA, and with that of ssOct generated from CTH and 50mer-ssDNA by using the standard gradient dialysis method¹² (see Methods). QELS

measurements showed that HD^x particles and the nucleosomes have a similar hydrodynamic diameter (Figure 2e). The QELS data also showed that the gradient dialysis generated a mixture of particles (PDI = 0.82, Figure 2f). The smaller particles lay in the hydrodynamic size regime similar to that of HD^x particles (~200 nm), whereas the larger particles have an average hydrodynamic diameter of 615 nm (Figure 2f). These larger agglomerates were formed, likely because of the long incubation period in high ionic strength buffers during gradient dialysis.¹²

More accurate size distributions were measured by TEM imaging of the HD^x particles and the nucleosomes. Negative staining TEM of nucleosomes and the HD^x particles generated from CTH or CTH^{-H1} showed high-contrast, well-resolved particles of diameter 10 –11 nm, which matches the expected size of a typical nucleosome (Figure 3a,b and Figure S8a,b). We note that we occasionally observed spots on control TEM grids in a different size regime compared with the HD^x particles (Figure S8c,d), so these spots did not interfere with our analysis. Longer ssDNA (150 nucleotides) also showed HD^x of a similar diameter of 10 –11 nm (Figure 3a). However, CTH-ssDNA complexes generated at a high ionic strength ($C_{\text{CTH}} = 240 \text{ nM}$, $C_{\text{ssDNA}} = 120 \text{ nM}$, 150 mM Tris buffer) or at a high concentration of the CTH ($C_{\text{CTH}} = 14.4 \text{ }\mu\text{M}$, $C_{\text{ssDNA}} = 7.2 \text{ }\mu\text{M}$, 1 mM Tris buffer) showed large nonspecific aggregates (Black arrows in Figure 3c-e). These aggregates are distinct from the HD^x particles (Figure 3b) and from CTH (Figure 8e,f). We also observed particles whose size is similar to the HD^x particles (White arrows in inset of Figure 3c and Figure. 3d,e), suggesting the partial formation of the HD^x particles at these conditions. Cryo-TEM images of the HD^x particles showed that 46 % of the particles lay within the size regime observed by using negative staining and the expected size distribution of nucleosome (Figure 3f,g), whereas larger particles with average diameter of 20 nm constituted 54 % of the HD^x particles (see Note S2). The formation of well-isolated particles with diameters of 10 nm and 20 nm (Figure 3b,g) provides

strong evidence that rules out the formation of large nonspecific aggregates at the conditions in which the HD^x particles are most efficiently formed ($C_{CTH} = 240 \text{ nM}$, $C_{ssDNA} = 120 \text{ nM}$, 1 mM Tris buffer, Figure 3a,b,g).

Figure. 3. Characterization of the physical size of the self-assembled histone-ssDNA nanostructures by using electron microscopy imaging. a) Size distribution of the HD^x particles generated at the 1 mM concentration of Tris buffer by using CTH ($C_{CTH} = 24 \text{ nM}$) and 50mer-ssDNA ($C_{ssDNA} = 120 \text{ nM}$) (top), CTH^{H1} ($C_{CTH}^{H1} = 24 \text{ nM}$) and 50mer-ssDNA ($C_{ssDNA} = 120 \text{ nM}$)

(middle), and CTH ($C_{\text{CTH}} = 24 \text{ nM}$) and 150mer-ssDNA ($C_{\text{ssDNA}} = 120 \text{ nM}$) (bottom). Insets: TEM image of HD^x particles. b) Enlarged view of the HD^x particles generated by using CTH^{H1} ($C_{\text{CTH}}^{\text{H1}} = 24 \text{ nM}$) and 50mer-ssDNA ($C_{\text{ssDNA}} = 120 \text{ nM}$) (white arrow). c) TEM micrograph of CTH-ssDNA generated at a high ionic strength ($C_{\text{CTH}} = 240 \text{ nM}$, $C_{\text{ssDNA}} = 120 \text{ nM}$, 150 mM Tris buffer). Inset: Enlarged view. d) TEM micrograph of CTH-ssDNA generated at a high concentration of the CTH ($C_{\text{CTH}} = 14.4 \text{ }\mu\text{M}$, $C_{\text{ssDNA}} = 7.2 \text{ }\mu\text{M}$, 1 mM Tris buffer). e) Enlarged view of the particles generated at the conditions identical to Figure 3d. The black and white arrows in c, d and e show the large nonspecific aggregates and the HD^x particles, respectively. f) Cryo-TEM image of CTH. Inset (left) is an enlarged view of the particles. Inset (right): Size distribution of the CTH particles. g) Cryo-TEM image of HD^x particles generated by using CTH and 50-mer ssDNA ($C_{\text{CTH}} = 240 \text{ nM}$, $C_{\text{ssDNA}} = 120 \text{ nM}$, 1 mM Tris buffer). Inset (top): Size distribution of the HD^x particles obtained from the Cryo-TEM images. The black line is the Gaussian fit of the size distribution of canonical nucleosomes shown in Figure S8b. The black shading highlights the frequencies of the HD^x particles that lie under the Gaussian fit. The white arrow indicates a particle (diameter = 11 nm) that lay in the same size regime like that of nucleosome (Supplementary Figure S8a,b). Inset (bottom) is an enlarged view of a 40-nm sized particle.

To elucidate the structure of the HD^x particles, we first compared the gel migration behavior of HD^x particles with that of nucleosome. To that end, native PAGE migration of nucleosomes and HD particles (generated by mixing Cy3-labeled 50mer-ssDNA and CTH) was realized on a polyacrylamide gel. Since we were not able to detect the proteins by gel electrophoresis at the 120

nM and 240 nM concentrations of 50mer-ssDNA and CTH respectively (HD^x particles), we conducted the gel experiments at 60 times higher concentrations of 50mer-ssDNA (7.2 μ M) and CTH (14.4 μ M) (denoted HD^{60x} particles). The native PAGE electrophoresis showed that the negatively charged nucleosome migrated to the positive electrode²¹ (Figure 2e). In contrast, we observed the migration of neither the HD^{60x} particles nor the Cy3-labeled DNA to the positive electrode (Figure S9a, c, see Note S3a for a clarification of this behavior). Instead, we observed the separation and migration of H1 toward the negative electrode (Figure 2e and Figure S9b,d). These results indicate that ssDNA associated with the HD^{60x} particles whereas H1 remains free in the solution (see Conclusions for a suggested interpretation of this behavior).

To obtain the stoichiometry of histone monomers in the HD^{60x} particles, we fractionated the HD^{60x} particles (that comprise particles with similar size to that of HD^x particles and large aggregates (Figure 3d,e)) by sucrose gradient fractionation followed by SDS-PAGE. Band quantification analysis following the SDS-PAGE migration of the fractions showed equal stoichiometry of the core histones in 45 % of the fractions (Figure S10 and Figure S11). This abundance is in close approximation to the relative abundance of the HD^x in the HD^{60x} particles (Figure S12). The remaining fraction (55%) that showed unequal stoichiometry can be attributed to non-specific aggregates formed at the high concentration (Figure 3d,e and Figure S12; see Note S3b for the performance of sucrose-gradient at our experimental conditions).

Having investigated the physical size and the stoichiometry of histone monomers, we attempted to obtain the stoichiometry of the ssDNA oligomers in the HD^x particles. To that end, we determined the number of 50mer-ssDNA molecules associated with single HD^x particles by means of single-particle fluorescence intensity and photon coincidence measurements (Figure S13). To label the ssDNA and to tether the HD^x particles to a neutravidin-coated coverslip, we attached a

Cy3 dye and biotin at 5' and 3' ends of 50mer-ssDNA, respectively (Cy3-50mer-ssDNA-biotin, Figure S14a). We then incubated the Cy3-50mer-ssDNA-biotin ($C_{\text{ssDNA}} = 120 \text{ nM}$) with CTH ($C_{\text{CTH}} = 240 \text{ nM}$) for 12 min to obtain HD^x particles (Figure S14b). Single-particle fluorescence intensity time-trajectory of the surface-tethered HD^x particles (see Methods and equation 1) showed multi-step photobleaching behavior (Figure S14c), which suggested the presence of several Cy3 dyes in the single HD^x particles. Importantly, photon correlation statistics obtained from the highest intensity level demonstrated that 40 % of the HD^x particles, generated after the interaction of 50mer-ssDNA with either CTH or CTH^{H1}, contained 6 Cy3 molecules (*i.e.*, six 50mer-ssDNA molecules (total 300 nt), Figure 4a,b and Figure S14d). We found that the HD^x particles generated from 100mer- and 150mer-ssDNA contained 3 Cy3 (*i.e.*, three 100mer-ssDNA molecules (total 300 nt), Figure 4c) and 2 Cy3 molecules (*i.e.*, two 150mer-ssDNA molecules (total 300 nt), Figure 4d), respectively.

Figure. 4. Characterization of the number of ssDNA molecules associated with sHD particles by using photon correlation statistics. a) Frequency histogram of the number of emitters in each HD^x particle estimated from the photon coincidence measurement. Inset: Fluorescence image of surface-tethered CTH-Cy3-50mer-ssDNA-biotin complexes ($C_{\text{ssDNA}} = 120$ nM, $C_{\text{CTH}} = 240$ nM, $T = 4$ °C, $t = 12$ min). The number of ssDNA molecules is $n = 6$. Scale bar = 2 μ m. b) Frequency histogram of the number of emitters in each HD^x particle generated from CTH^{-H1} and 50-mer ssDNA. The number of ssDNA molecules is $n = 6$. c) Frequency histogram of the number of emitters in each HD^x particle generated from CTH and 100-mer ssDNA. The number of ssDNA molecules is $n = 3$. d) Frequency histogram of the number of emitters in each HD^x particle generated from CTH and 150-mer ssDNA. The number of ssDNA molecules is $n = 2$.

Taken together, our results demonstrate the self-assembly of histone-ssDNA nanostructures that have an average size of 10-nm, equal stoichiometry of histone monomers and a constant number of 300 nucleotides. Because the assembly of the ssDNA-wrapped histone octamers (ssOct) has been assumed to follow different route with entirely different conformation compared with typical nucleosome,^{11,12} it is most likely that we assembled a conformer (the 10-nm particles with 46 % relative abundance, denoted single histone-DNA complex (sHD), Figure 3b) that has similar fundamental structural features as those of ssOct. However, the detailed spatial arrangement of the histone proteins and ssDNAs inside sHD remains an open question. Since it is well known that the tails of long ssDNA that dangle from one octamer associate with other octamers to form cross-linked octamers (CLO),¹² the observed larger particles in Figure 3g are attributed to cross-linked sHD (denoted cHD) (see below for detailed structural characterization of sHD and cHD using AFM).

Length dependence of the assembly of CTH by ssDNA

To investigate effects of the length of ssDNA on the self-assembly process of the HD^x (sHD and cHD) particles, we covalently labelled ssDNA oligomers (6 – 150mer) with a single self-quenching Cy5 fluorescent dye (Cy5-ssDNA).²² The self-assembly of CTH by Cy5-ssDNA causes spatial confinement of Cy5 dyes, and thus, would lead to fluorescence self-quenching, depending on the nanoscale spatial arrangement of Cy5-ssDNA in the histone-ssDNA complexes (Inset of Figure 5a and Figure S15).

Upon adding CTH to a solution of Cy5-ssDNA of different length, we observed fluorescence quenching of the Cy5 tags because of their proximal confinement in the sHD and the cHD particles (Figure 5a and Figure S16). In contrast, upon adding CTH to Cy5-50mer-ssDNA in 150 mM ionic strength buffer or upon adding CTH denatured with 4% perchloric acid to Cy5-50mer-ssDNA in 1 mM Tris buffer, we observed inefficient quenching of the Cy5 tags (Figure 5b). This indicated that the interaction mainly generated aggregates (Figure 3c shows the aggregates formed in high ionic strength buffer) rather than HD^x particles. The significant fluorescence quenching (Figure 5a) was observed at molar ratios of CTH and Cy5-ssDNA in which HD^x particles are formed. Maximum quenching (*i.e.*, maximal confinement of the Cy5 tags) occurred at a well-resolved histone:ssDNA fluorescence quenching ratio (ratio = 2.43 ± 0.086 for Cy5-50mer-ssDNA, Figure 5a and Figure S16), which we denote quenching cutoff ratio (QCR). An analogous fluorescence quenching profile with similar QCR value was observed for Cy5-ssDNA-CTH^{H1} complexes (Figure S17), further supporting the irrelevance of H1 to the assembly of HD^x particles. Since Cy5-ssDNAs are supposed to be confined at highest density in the HD^x particles at the QCR, the QCR obtained for different lengths of ssDNA oligomers can be used to investigate the length dependence of the assembly of HD^x particles.

The HD^x particles reconstituted from CTH and Cy5-labeled ssDNA oligomers of varying lengths showed ssDNA-length-dependent QCR (Figure 6a) and quenching efficiency (Figure 6b). Importantly, a plot of the QCR of different *n*-mers and the magnitude of quenching at each QCR revealed an exponential dependence on oligomer length that differentiated between single-nucleotide differences for longer ssDNA oligomers (Figure 6a). The 6mer-ssDNA deviates strongly from this trend (Figure 6b). QELS measurements on CTH-6mer-Cy5-ssDNA at the QCR showed the onset of size-regimes centered at 50 nm and at 800 nm (Figure 6c) that strongly deviate

from the expected size of HD^x particles. This result demonstrates the inability of 6mer-ssDNA to organize CTH into well-defined HD^x particles. As the length of ssDNA increased to 15nt, the quenching efficiency at the QCR markedly increased (red dashed arrow in Figure 6b) with a concomitant increase in the histone:ssDNA QCR (Figure 6a). The QELS measurements demonstrated the formation of HD^x particles at this condition (Figure 6c) with relatively broad size distribution (Figure 6d). As the length of ssDNA increased past 15nt and up to 100nt, the fluorescence quenching experiments showed the increased QCR (histone:ssDNA ratio, Figure 6a and Figure S18a) and decreased fluorescence quenching magnitude (Figure 6b). The observations are consistent with a smaller number of ssDNA molecules and hence a lower average density of self-quenching dyes around a histone octamer (Figure 4). The 150mer-Cy5-ssDNA did not show fluorescence quenching of the Cy5 tags (Figure S18b). This observation is consistent with the presence of only two Cy5-labeled ssDNA molecules in the sHD particles (Figure 4d), and hence the Cy5 tags are not in close enough proximity to allow quenching to occur. Interestingly, the least polydisperse HD^x particles generated from 150mer-ssDNA and CTH were obtained at histone:ssDNA ratio of 16, which agrees with the expected QCR ratio of this ssDNA length (Figure S18c,d). Concomitant with the increase of the length of Cy5-ssDNA from 15nt to 35nt, narrower size distribution of HD particles was observed (Figure 6c,d). QELS data of histone-ssDNA complexes using ssDNA with lengths between 50-150nt were very similar to each other in the predominance of HD^x particles with small PDI at the QCR and were indistinguishable based on QELS peak positions (Figure 6c) and polydispersity (Figure 6d).

Figure 5. Characterization of the formation of HD^x particles using fluorescence self-quenching.

a) Spectrally integrated fluorescence intensity of Cy5-50mer-ssDNA ($C_{\text{ssDNA}} = 120 \text{ nM}$) upon mixing with fixed amounts of CTH ($C_{\text{CTH}} = 24 - 2.4 \text{ } \mu\text{M}$). QCR denotes quenching cutoff ratio. Inset: a schematic illustration of the co-localization of ssDNA around HD particle, and resultant self-quenching of Cy5 fluorescence by Cy5 dimer (double arrow). Cy5 tags are shown in red. b) Spectrally integrated fluorescence intensity of Cy5-50mer-ssDNA ($C_{\text{ssDNA}} = 120 \text{ nM}$) upon mixing with fixed amounts of non-denatured CTH ($C_{\text{CTH}} = 24 - 2.4 \text{ } \mu\text{M}$) in 1 mM Tris buffer supplemented with 150 mM NaCl and with CTH denatured with 4% perchloric acid.

Figure 6. Length dependence of the histone-ssDNA interaction. a) Change in QCR as a function of ssDNA length. The red line is an exponential fit serving as a guide for the eye ($C_{\text{ssDNA}} = 120$ nM, $C_{\text{CTH}} = 63, 100, 145, 170, 177, 193, 292, 331, 335,$ and 411 nM for 6, 15, 25, 30, 32, 35, 50, 55, 56, and 65mer-ssDNA, respectively). Error bars show the standard deviation of three independent measurements. b) Fractional fluorescence intensity at the QCR as a function of ssDNA length ($C_{\text{ssDNA}} = 120$ nM, $C_{\text{CTH}} = 63, 100, 145, 170, 177, 193, 292, 331, 335,$ and 411 nM

for 6, 15, 25, 30, 32, 35, 50, 55, 56, and 65mer-ssDNA, respectively). Insets: schematic figures illustrating histone-ssDNA complexes obtained with 6mer-, 25mer- and 50mer-ssDNA. c) Contour plot illustrating the hydrodynamic diameter of different CTH-ssDNA complexes at the QCR as a function of ssDNA length. The green dashed line points to the ssDNA length of 15 nt. The red dashed line points to the ssDNA length of 50 nt. d) Polydispersity indices (PDI) of CTH:ssDNA complexes as a function of ssDNA length ($C_{\text{ssDNA}} = 120$ nM, $C_{\text{CTH}} = 63, 100, 145, 193, 292, 331, 335,$ and 411 nM for 6, 15, 25, 35, 50, 55, 56, and 65mer-ssDNA, respectively). The plot illustrates the exponential decrease in PDI of the particles at the QCR upon increasing the ssDNA length. Error bars show the standard error of the mean. e) Biphasic dependence of the ratio of experimental (ssDNA^{HD}) and the calculated ($\text{ssDNA}^{\text{oct}}$) number of ssDNA molecules associated with histone complexes on the length of ssDNA.

Characterization of two distinct CTH-ssDNA complexes: sHD and cHD.

The detailed characterization of HD^{x} particles obtained from 50mer-ssDNA and CTH demonstrated the co-existence of 10-nm particles (sHD, 46 %) that have similar structural features as that of ssOct and larger particles (cHD, 54 %) that represent the cross-linked complexes of sHD (Figure 3g). To provide closer structural insights into these complexes, we calculated the stoichiometry of the ssDNA oligomers in the sHD and cHD particles using a geometric accommodation model. We, first, calculated the average total number of 50mer-ssDNA molecules associated with the HD^{x} particles by using the experimentally determined histone:ssDNA molar ratio at the QCR (assuming that a typical octamer is assembled without cross-linking). We found

that this experimentally determined number *via* QCR (ssDNA^{HD} (*i.e.*, sHD and cHD)) = 3.7 molecules, Table S1) is much smaller than the other experimentally determined number of ssDNA associated with a single sHD ($\text{ssDNA}^{\text{sHD}} = 6$ molecules, Figure 4a,b; note that this experimental value coincides well with the calculated total number of 50mer-ssDNA molecules associated with one typical octamer (calculated $\text{ssDNA}^{\text{oct}} = 6.2$ molecules, see Table S1 and Methods)). Thus the difference between the values of ssDNA^{HD} (*i.e.*, number of ssDNA associated with both sHD and cHD) and $\text{ssDNA}^{\text{sHD}}$ indicates that cHD originate with different stoichiometry. We further confirmed this difference in stoichiometry by calculating the number of ssDNA associated with a single cross-linked sHD particle in cHD particles ($\text{ssDNA}^{\text{cHD}}$) by using the relative abundance of sHD and cHD (46 and 54 %, respectively), and the experimentally determined number of ssDNA associated with single sHD ($\text{ssDNA}^{\text{sHD}}$) and HD^x (ssDNA^{HD}) (see Methods). We found that the $\text{ssDNA}^{\text{cHD}}$ value is significantly smaller than the $\text{ssDNA}^{\text{sHD}}$ ($\text{ssDNA}^{\text{cHD}} = 1.7$) and coincides well with the expected number of 50mer-ssDNA molecules that could wrap around an octamer along their contour lengths without loops (1.5 molecules, denoted single-copy wrapping). The large histone-ssDNA complex (cHD) is, thus, stoichiometrically different from the smaller histone-ssDNA complex (sHD) that contains 6 molecules of 50-mer ssDNA (Figure 4a). These findings suggest that cHD is stoichiometrically distinct from sHD and this distinction could explain the single nucleotide sensitivity of our fluorescence quenching experiment (see Note S4).

To provide another proof that cHD is stoichiometrically distinct from sHD, we determined the experimental ssDNA^{HD} and the calculated $\text{ssDNA}^{\text{oct}}$ for ssDNA with different lengths and then plotted experimental ssDNA^{HD} :calculated $\text{ssDNA}^{\text{oct}}$ ratio *versus* ssDNA length (*n*-mers) (Figure 6e). A large experimental ssDNA^{HD} :calculated $\text{ssDNA}^{\text{oct}}$ ratio corresponds to the efficient formation of sHD over cross-linked states including cHD. As the length of ssDNA increased from

6 to 35mers, the experimental ssDNA^{HD}:calculated ssDNA^{oct} ratio also increased (Figure 6e). This result suggests that the longer ssDNA promotes the formation of structurally organized sHD, consistent with the results obtained from the QELS measurements (Figure 6c,d). When the length of ssDNA further increased from 35 to 150mers, a decrease in the experimental ssDNA^{HD}:calculated ssDNA^{oct} ratio was observed (Figure 6e). This decrease suggests that cHD (containing lower number of ssDNA molecules per a cross-linked sHD) is formed more efficiently with longer ssDNA in this length regime. Together, our analysis suggests the ssDNA-length-dependent ordered progression of the histone:ssDNA self-assembled complexes from the non-specific aggregates (6mer) to sHD (15 to 35mers) and ending with sHD and cHD (50 to 150mer).

To provide a direct experimental elucidation that cHD originates with a distinct stoichiometry of ssDNA, we used Cy3-50mer-ssDNA to generate HD^x particles. We then tracked the electrophoretic velocities (v) of single HD^x particles and measured their fluorescence intensity (A) inside a capillary channel filled with 0.2% agarose (Figure 7a, see Methods). We note that v is proportional to the charge to radius ratio (q/r) of the particles in a uniform electric field ($E = 5 \text{ V cm}^{-1}$) by $v = c \cdot q/r$, where c is a constant under our experimental conditions ($c = E/6\pi\eta$, η denotes the viscosity of the media).²³ We also note that A is proportional to the number of Cy3 molecule (N), *i.e.*, the number of ssDNA in single particles. If the cHD complexes are formed by a simple assembly of the sHD complexes, the relationship between A and v would show linear dependence with a negative slope where $N \propto r/q$. However, our experimental results showed that the relationship between v and A is nonlinear and is best fitted by a piecewise linear regression with two slopes and a breakpoint (Figure 7b). The particles with faster velocity ($v = 34 \pm 3 \text{ } \mu\text{m s}^{-1}$) showed insignificant variation in the fluorescence intensity (slope approaches zero; slope = -0.006) and are attributed to the sHD particles that contain fixed number of ssDNA ($n = 6$, Figure 4a). On

the other hand, the particles with slower velocity ($v = 25 \pm 3 \mu\text{m s}^{-1}$) showed a significant decrease in the fluorescence intensity as v increases (slope = -0.06) and are attributed to the cHD complexes. The steeper slope obtained from cHD indicates a larger net positive charge (q) compared with sHD and hence the cHD complexes contain smaller number of ssDNA molecules per a cross-linked sHD. These results are qualitatively consistent with the estimated molar ratio between ssDNA and histones in sHD and cHD (6 and 1.5 molecules, respectively) and strengthen our finding that the stoichiometrically-distinct sHD and cHD complexes are self-assembled at our optimized assembled conditions.

Figure. 7. Characterization of two stoichiometrically distinct sHD and cHD particles by using electrophoretic single particle tracking. a) Time-lapse fluorescence images of the migration of a single HD^x particle toward the negative electrode. We note that the electroosmotic flow complicates the electrophoretic migration in our experiment and thus it is difficult to drive conclusive evidence on the charge of the particles based on the direction of migration. Scale bar = 1 μm . b) Normalized fluorescence signal amplitude of HD^x particles as a function of the velocity of electrophoretic migration. The red line is a fit to a piecewise linear regression function.

Self-assembly of histone-DNA nanostructures on a solid surface.

We next investigated self-assembled structures of ssDNA and CTH deposited on a solid surface using 65mer-ssDNA. According to the geometric accommodation model, the single-copy wrapping of this ssDNA would spans 12 binding sites, and therefore it could conceivably bridge the 8 histone monomers (Figure 1b and Figure 8a). Self-assembled structures on a mica surface were characterized by means of dry-phase AFM. At a histone:ssDNA ratio of 2 (*i.e.*, below QCR), we observed single particles of 3-4 nm height deposited on the surface (Figure 8b). This height agrees with our measurement on nucleosomes (Figure S19) and the consistently-reported height of ssOct measured from dry phase AFM images.^{11,24,25} Therefore, these particles represent the sHD particles. We also observed mono- and multi-layered cross-linked assemblies that represented a transition between individual sHD (Figure 8c,d) and their cross-linked 2D (Figure 8c and Figure 9a), two-layered 3D (Figure 8c and Figure 9b), three-layered 3D (Figure 8d and Figure 9c,h) and multi-layered 3D (Figure 8e and Figure 9d) nanostructures. Therefore, these particles represent the cHD particles. A close look at the periphery of these cross-linked nanostructures revealed parallel ssDNA pairs (single copies of approximately 1 nm height and a few nm lateral distance) dangling from the cross-linked 2D particles (Figure 9e,i). The ordered 2D and 3D nanostructures demonstrate the formation of multidimensional self-organized nanostructures on the surface.

At a histone:ssDNA ratio of 3.4 (*i.e.*, at QCR), the self-assembly generated particles of two distinct size regimes. The first regime had a height of 4 nm and is consistent with the height of nucleosome (Figure 8b and Figure S20), whereas the second regime had a mean size of 17 nm, suggesting that more sHD were further assembled to the transient 2D and 3D structures (Figure 8c,d) to form multi-layered (x) 3D nanostructures (cHD_x). The sparse formation of cHD_x particles at ratio 2 (Figure 8e) and the absence of the transient cHD structures at ratio 3.4 suggest that the

assembly started at ratio 2 and is complete at the ratio 3.4 (Figure 8b). We also observed occasional assembly of multiple sHD to form 1D nanostructures (Figure 9f,g,i (inset) and Figure S21). These fiber-like 1D nanostructures (chromatin-like²⁶) showed equally-spaced sHD interconnected by single 65mer-ssDNA. At a histone:ssDNA ratio of 9 where histones are in excess, the interaction generated particles of mean height of 2 nm. These particles are smaller than sHD and most likely represent CTH monomers/dimers and their non-specific assemblies with ssDNA.

Figure. 8. Self-assembly of histone-ssDNA nanostructures on solid surface. a) The geometry of the contour length of a 65-mer polythymine (T) ssDNA is shown with respect to the SHL -6.5 to 6.5. b) AFM height distribution of the particles generated after the interaction of 65mer-ssDNA (120 nm) and CTH at histone:ssDNA ratio of 2 (pre-QCR), 3.4 (QCR) and 9 (post-QCR). Insets: AFM topographic images of the generated particles at each ratio. The color bar shows the corresponding fluorescence intensity as shown in Figure S16f. c-e) AFM topographic images of the generated 2D and 3D assemblies at ratio of 2. cHD_{x1}; denote cHD particle with x1 cross-linked sHD arranged in a single layer. cHD_{x1,x2}; denote cHD particle with x1 and x2 cross-linked sHD

arranged in layer 1 and layer 2 respectively. $cHD_{x1,x2,x3}$; denote cHD particle with $x1$, $x2$ and $x3$ cross-linked sHD arranged in layer 1, layer 2 and layer 3 respectively. cHD_{xm} ; denote cHD particle with m cross-linked sHD arranged in multi-layers. The red arrows indicate histone sHD. By using a simple volume filling algorithm, the cHD_x particles had an average of 300 smaller sHD (cHD_{x300}). The dashed white square in Figure 8b illustrates a cHD_{x560} particle, whereas the dashed yellow square represents a cHD_{x330} particle.

Figure 9. Mechanism and kinetics of self-assembly of histone-ssDNA nanostructures. a-d) AFM topographic images (left), their 3D height (middle) images, and schematic illustrations (right) of (a) cHD₄₄ (denote 44 sHD arranged in a single layer of cHD), (b) cHD_{37,5} (denote 37 and 5 sHD arranged in layer 1 and layer 2, respectively), (c) cHD_{22,7,2} (denote 22, 7 and 2 sHD arranged in layer 1, 2 and 3 respectively, see Figure 9h for the height profile of the red dashed line), and (d) CIO_{x330} (denote 330 sHD arranged in multiple layers (x layers)) particles. The number of sHD is calculated based on simple volume filling. The red arrows in Figure 9d (right) indicate more sHD in a layer (horizontal arrow) and indicate more sHD in multilayers (vertical arrow). e) AFM

topographic images (left) and a schematic illustration (right) showing two sets (arrowheads) of parallel ssDNA dangle from a cHD particle. The dashed blue line serves as a guide for the eye. f) AFM topographic image (left) of deposited nanostructures at the QCR. g) An enlarged view of the white square in Figure 9f displaying a 1D histone-ssDNA structure. h) Height profile of the cHD particle shown in Figure 9c. i) Height profile of the ssDNA that dangle from the cHD particle shown in Figure 9e. Inset: height profile of the ssDNA that extend from the sHD particle of a larger cHD particle shown in Figure 9e (right). The inset has the same axis title as in Figure 6i. j) Schematic illustration of the proposed kinetic model of histone-ssDNA self-assembly mechanism.

CONCLUSIONS

In this study, we demonstrated that the interaction of ssDNA and CTH at exceedingly low concentration and ionic strength (1 mM Tris buffer, $C_{\text{ssDNA}} = 120 \text{ nM}$, $C_{\text{CTH}} = 240 \text{ nM}$, $T = 4 \text{ }^{\circ}\text{C}$ and $t = 12 \text{ min}$) generated HD^x particles with small PDI (Figure 2) that show physical size and stoichiometry of histone monomers and ssDNA bases similar to those of the histone octamer wrapped by ssDNA (ssOct) (Figure 3, Figure 4 and Figure S11). Strikingly, we showed that regardless the length of ssDNA, up to 50 % of the HD^x particles contain on average 300 nt (Figure 4), which is in very close approximation to the number of dsDNA bases (147 bp = 294 nt) contained in one typical nucleosome. Therefore, our results (Figure 2, Figure 3 and Figure S11) suggest that we generated a fundamental building block particles (sHD particles) that are structurally similar to the double-copy-wrapped ssOct (see Note S5 for a proposed pathway that could explain this self-assembly).

CTH and CTH^{H1} generated HD^x particles of the same physical size (Figure 3a) and the same number of DNA bases (Figure 4a,b) and showed identical fluorescence quenching behavior (Figure S17). These results point to an irrelevant role of H1 in the assembly process where H1 remains free in the solution (Figure S9, see below for a suggested interpretation of this behavior). In contrast to the assembly of low-polydisperse HD^x particles by ssDNA and CTH (or CTH^{H1}), the mixture of RecH and ssDNA did not generate self-assembled HD^x particles with small PDI (Figure 2a, Figure S5 and Figure S6).

The detailed analysis of the ssDNA-length-dependent assembly of HD^x particles revealed a key factor for the successful self-assembly of ssDNA and CTH. The fluorescence self-quenching experiment suggested that HD^x particles are not self-assembled by the short 6mer ssDNA while the HD^x particles are generated by the slightly longer 15mer-ssDNA (Figure 6b,c). According to the hypothesized geometric accommodation model, the contour length of 6mer-ssDNA (4.2 nm) is barely sufficient to span only two docking sites (4 nm spacing, Figure 1b). On the other hand, the 15mer-ssDNA with the contour length of 10.5 nm spans three docking sites across two monomers (Figure 1b). Our findings, thus, suggest that the bridging of three docking sites by ssDNA is the key step for the formation of HD particles.

One of the key findings is the self-assembly of the structurally-distinct ssDNA-histone complexes, sHD, and cHD. Our characterization demonstrated that the sHD complex is structurally similar to ssOct whereas the single-copy-wrapped cHD (C1O-like complex) is stoichiometrically distinct from sHD (Figure 6e and Figure 7). For more detailed structural characterization of these nanostructures including the spatial arrangement of histone monomers and ssDNA within sHD and cHD, the employment of other techniques such as X-ray crystallography or HRTEM single particle analysis would be necessary.

Our observations provide a clue to the kinetics of the formation of sHD and cHD (Figure 9j). Since none of sHD displayed dangled ssDNA, we posit that sHD and cHD are not interchangeable once they are formed. The presence of the intermediate 1D nanostructures with equally-spaced sHD (Figure 9g) suggest that cHD is formed through the crosslinking of sHDs. Taken together, we posit that a precursor single-copy-wrapped octamer is initially formed. This precursor is converted to either the double-copy-wrapped isolated sHD by the additional binding of ssDNAs to the octamer or the single-copy-wrapped cHD by bridging them with ssDNAs. This single-copy wrapping along with our geometric accommodation model can explain the single-nucleotide-resolution fluorescence quenching of histone-ssDNA interaction (Note S4). Furthermore, because the precursor single-copy-wrapped octamer contains less mass of DNA compared with a typical nucleosome, it is likely that this precursor bears a positive charge. Therefore, we posit that the charge repulsion that could originate between the lysine-rich H1 and this precursor explains the irrelevance of H1 to the self-assembly.

The self-assembly of single-copy-ssDNA-wrapped histones demonstrated in this study provides essential implication towards geometry-based DNA-protein self-assembly. Because the wrapping of DNA around histone proteins proceeds with precise geometries (*i.e.* 10 nm fixed size associated with 300 nt), one could control the self-assembly based on the number of ssDNA molecules to build multidimensional histone-DNA nanostructures (Figure 8 and Figure 9). We believe that our geometry-based self-assembly can be used in various design principles of histone-DNA hybrid nanomaterials. For example, the histone-ssDNA self-assembled nanostructures reported in this study can be used as building blocks of DNA-based self-assembling structures that require long-range structural programming at the single-nucleotide level. The single-copy ssDNA-wrapped cHD with the dangled sticky tails (Figure 9e,g, and Figure S21) can also be exploited in a DNA-

histone origami design. Alternatively, dangled ssDNA tails of a DNA origami motif can be used to self-assemble a histone octameric structure. Because the length of these tails determines the number of the motifs that can attach to this octameric structure, complex 2D and 3D histone-DNA structures could be designed by programming the length of the ssDNA tails (*i.e.*, geometry-based design) rather than their sequences. Overall, our assembly platform can offer promising opportunities for broader applications in the field of DNA-protein nanotechnology.

MATERIALS AND METHODS

Materials. Polythymine dye-free and Cy5-labelled oligonucleotides were purchased from Integrated DNA Technologies (IDT, Leuven, Belgium) and from Nihon Gene Research Laboratories (NGRL, Tsukuba, Japan). A natural mixture of calf thymus histone monomers (CTH; mixture of H1, H2A, H2B, H3, and H4) was purchased from Roche Diagnostics (Indianapolis, IN, USA). The isolation is performed without any heat steps or detergents. The native histones are separated from DNA by adding 0.9 volumes of 5 M NaCl and then the salt is removed by dialysis. To further remove the residual salt from the commercial preparation, we reconstituted CTH in 1 mM TE buffer at concentration of 1 mg ml⁻¹ and then the desalting was done by using Vivaspin[®] centrifugal concentrator (Hydrosart[®], 10,000 MWCO, Vivaproducts, MA, USA). Typically, 400 µl were introduced into the concentrator and then spun at 3,000 g for 2 min. The remaining 200 µl was then diluted with 1.8 ml of 1 mM TE buffer and then centrifuged at 3,000 g for 5 min. The concentrator was then refilled with fresh TE buffer and the process of desalting was repeated 5 times. After the desalting step, CTH was quantified by measuring the absorbance at 230 nm. It is important to underline that we routinely used the fluorescence quenching profile (Figure 5a) to judge the quality of the histone preparation. Distortion of this profile indicates the presence of

residual salt in the solution (above 1 mM) or denatured CTH (Figure 5b). Human recombinant histone monomers, H1, H2A, H2B, H3, and H4, were purchased from New England BioLabs (Ipswich, MA, USA). Human recombinant nucleosomes were purchased from Epigex (Strasbourg, France) and used as a control for QELS and TEM experiments.

Preparation of Histone H1-depleted CTH. Histone H1 was removed from CTH by batch ion-exchange extraction.²⁷ The resin AB50W $\times$ 2 (2% crosslinking, BioRad, USA) used in our study was washed in 3 $\times$ 5 volumes of 1M HCl followed by 3 $\times$ 5 volumes of milliQ water. The resin was then activated by washing in 3 $\times$ 5 volumes of 1M NaOH followed by 3 $\times$ 5 volumes of milliQ water. One ml of CTH histone solution at 1mg ml⁻¹ in phosphate buffer (50mM sodium phosphate, 0.45M NaCl, 0.1 mM PMSF, pH = 7), was then added to 1 ml resin at 38 mg ml⁻¹. The mixture was shaken gently at 4 °C for 90 minutes. The resin was then decanted, and the supernatant was dialyzed against 1mM TE buffer. The resin showed higher affinity to bind H1 compared with histones H2A-H2B and H3-H4 because of its high lysine content. The efficiency of H1 depletion was tested using SDS-PAGE (Figure S1b).

Estimation of the concentration of H1-depleted CTH. The concentration of H1-depleted histone was estimated by using NanoOrange[®] Protein Quantification Kit (Molecular Probes, OR, USA) as described in the protocol accompanying the kit. Serial dilutions of known concentrations of CTH were used to construct the calibration curve for estimating the concentration of CTH^{H1}.

Reconstitution of Histone-ssDNA Complex. Solutions of Cy5-5'-ssDNA (120 nM, 50 μ l each) in 1 mM TRIS buffer (pH = 7.5) were individually mixed with 150 μ L of CTH in 1 mM TRIS buffer (pH = 7.5) at various molar concentrations (60 nM ~ 2400 nM) to prepare solutions at each measured histone:ssDNA ratio. The process of independent mixing, rather than serial additions to

the initial solution, was chosen to eliminate the possibility of persistence of any structures formed in an earlier mixture of ssDNA and histone at a different concentration ratio.

Reconstitution of Histone Octamers Using Gradient Dialysis. Solutions of Cy5-5'-ssDNA (120 nM, 125 μ l each) in 1 mM Tris buffer (pH = 7.5) were individually mixed with 375 μ l of CTH in 1 mM Tris buffer (pH = 7.5) at histone:ssDNA ratio 2. The Tris buffer, used for the reconstitution, was supplemented with 2 M NaCl to promote the interaction of the histone subunits. The 500 μ l solution was then subjected to successive, rapid dialysis against a series of Tris buffers containing decreasing amounts of NaCl. The NaCl concentration is decreased in steps of 0.2 M every 90 min from 1.5 to 0.6 M NaCl. The solution was then finally dialyzed against 1 mM Tris buffer (pH = 7.5) for 24 h at 4 °C.

Quasielastic Light Scattering (QELS). Solutions of Cy5-5'-ssDNA (120 nM, 50 μ l each) in 1 mM TRIS buffer (pH = 7.5) were individually mixed with 150 μ L of CTH in 1 mM Tris buffer (pH = 7.5) at various molar concentrations (60 nM ~ 600 nM) to prepare solutions at each measured histone:ssDNA ratio. The minimal time between mixing and measurement is 12 minutes to minimize the possibility of aggregation. QELS data were acquired using Malvern Zetasizer Nano ZS (Malvern, Worcestershire, United Kingdom). The system was equipped with a HeNe laser (633 nm) and was capable of measuring the size of particles between 0.3 nm to 10 μ m. Data were analyzed using Zetasizer (Malvern) and OriginPro (Originlabs) software. The polydispersity index of the original QELS peak was calculated using Zetasizer software. We recorded the QELS data from a single measurement (correlation time = 5 s) at different incubation times by preparing the histone:ssDNA mixed solution for each incubation time rather than averaging multiple data using the same solution to avoid the effect of photoirradiation-induced aggregations²⁸ of the histones and ssDNAs.

Fluorescence Spectroscopy. We used the same procedures of the reconstitution of histone-ssDNA complex to prepare 200 μ L solution at different histone:ssDNA ratios. The minimal time between mixing and measurement is 12 minutes. Fluorescence quenching studies were done using a spectrofluorometer FP-6300 (Jasco, Japan) and using a spectrofluorometer Fluoromax-4 (Horiba Scientific, Japan). Fluorescence spectra of Cy3 and Cy5 were measured upon excitation at 520 nm and 630 nm, respectively. The integrated fluorescence intensity was calculated in each case using OriginPro (Originlabs) software. The fractional fluorescence was calculated by (fractional fluorescence = integrated fluorescence intensity of ssDNA-Cy5 at the QCR / integrated fluorescence intensity of histone-monomers-free ssDNA-Cy5 solutions).

Single molecule tracking of the electrophoretic migration of histone-ssDNA complex. Sixty μ L of 0.2 % agarose solution were introduced into a capillary channel (17 mm $\times$ 3.8 mm $\times$ 0.4 mm) of ibidi sticky-slide IV (ibidi, Martinsried, Germany). One hundred μ L of histone-Cy3-labeled ssDNA complex ($C_{\text{TH}} = 0.24 \mu\text{M}$, $C_{\text{ssDNA}} = 0.12 \mu\text{M}$) in 1 mM Tris buffer (pH = 7.5) were then introduced at the loading well of the channel. A fixed voltage (5 V cm^{-1}) was then applied across the channel, and the electrophoretic migration²⁹ of the particles was tracked on a custom-built epifluorescence microscopy setup.^{30,31} The data were analyzed as described elsewhere.³²

Sucrose Gradient Sedimentation Assay. The sucrose gradient was prepared by layering less dense sucrose solutions upon one another to achieve a gradient of 5-20% sucrose concentration. The gradient was created using Gradient Master (BioComp Instruments, NB Canada) and was buffered with 1 mM TRIS buffer (pH = 7.5). 200 μ L of histone-ssDNA mixture ($C_{\text{TH}} = 14.4 \mu\text{M}$, $C_{\text{ssDNA}} = 7.2 \mu\text{M}$) in 1 mM Tris buffer (pH = 7.5) was placed over 5 ml of the 5-20 % sucrose density gradient and then centrifuged using Optima L-100K Ultracentrifuge with SW28 rotor (Beckman Coulter, CA, USA) at 4 $^{\circ}\text{C}$, 6000 RPM, for 6 h (see Note S3b). The gradient was then

fractionated into 22 fractions (230 μ l each) and then concentrated by centrifuging the individual fractions on Amicon Ultra 0.5 ml centrifugal filter (MWCO 3 kDa) at 14,000 g for 30 min. 13 μ l of each concentrated fraction were used for SDS-PAGE analysis.

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed using Novex 4-20 % Tris-Glycine gels in Tris-Glycine running buffer (Thermo Fisher Scientific Inc.). The native-page analysis was performed using Novex 4-20 % Tris-Glycine gels in Tris-Glycine native running buffer (Thermo Fisher Scientific Inc.). Protein bands were stained using Coomassie Blue (SimplyBlue™ SafeStain-Thermo Fisher Scientific Inc.). Images were obtained using Gel Doc EZ gel imaging instrument (Bio-Rad, Hercules, California, U.S.A.). Analysis of the gel image was carried out using OriginPro and ImageJ software where the peak intensity of the line profile of each band was used to compare the stoichiometry.

Atomic Force Microscopy (AFM) Imaging of Histone-ssDNA Complex. To conduct the dry phase AFM, a few microliters of the reconstitutes at the QCR were deposited on freshly cleaved mica surface, vacuum dried and, then, imaged using Olympus micro cantilever OMCL-AC160TS-C2 (Olympus, Japan) and a nanoscope IIIa MultiMode system (Digital Instruments, Santa Barbara, CA, USA). The number of HD particles and their height in each image was determined either manually or using a script written in Matlab. Images were processed and analyzed by using Gwyddion, Picoview, OriginPro, ImageJ and Matlab software. To analyze the AFM data, the original AFM scan files were imported into Gwyddion and then the AFM height images were exported as 8-bit grey-scale tiff files. We then constructed a height calibration curve with the grey values of the exported tiff files plotted against their corresponding Z-value (height) obtained from the original height images that we imported into Gwyddion. To calculate the distribution of the

particle size in the AFM images, we, first, used the local maxima algorithm to localize the particles and to export a list of their coordinates. This step was followed by finding the grey value of each coordinate and, then, the correct Z-values (height) from the height-calibration curve we constructed. This process was automated for all of the images using a home-written Matlab script.

Cryo-TEM Imaging of Histone-ssDNA Complex. Histone-ssDNA complex samples for Cryo-TEM were vitrified by ultra-rapid freezing. Typically, 2 μ l of the sample were put on a copper grid coated with holy carbon layer and blotted from both sides. The blotted grid was then immersed into liquid ethane using a Vitrobot (FEI company). The frozen grid was then loaded onto Gatan's side-entry cryo-transfer holder, model 626 whose temperature was kept at -192°C during the whole loading process. The holder was then loaded in the TEM instrument used for investigation of specimens. Imaging of samples was performed on a Titan G2 80–300 kV transmission electron microscope (TEM) from FEI Company equipped with a 4 k $\times$ 4 k CCD camera model US4000 and an energy filter model GIF Tridiem (Gatan, Inc.) or a Titan Krios equipped with a 2K CCD placed at the end of Gatan energy filter and operated at 300 kV. Typically, electron doses of $\sim$ 100 electrons $\text{\AA}^{-2}$ were used. Human recombinant nucleosome (0.2 mg ml⁻¹) was adsorbed on a carbon coated TEM grids (glow discharged) and negatively stained using 1 % uranyl acetate solution. The HD particles were adsorbed on ultrathin carbon film supported by a lacey carbon film on a 300 mesh gold grid (glow discharged). Imaging was performed by using a transmission electron microscope (TEM) operating at 300 kV (Titan Cryo Twin, FEI Company, Hillsboro, OR). Images were captured on a 2k x 2k CCD camera (Gatan Inc., Pleasanton, CA). The particle size was determined manually for negatively stained TEM images and as described elsewhere³³ for the Cryo-TEM images.

Fluorescence Photon Coincidence Experiment. Glass coverslips (0.17 mm thickness) were cleaned by sonication in ethanol (Sigma-Aldrich) for 15 min followed by 15 min sonication in 1 M KOH and then thoroughly rinsed with Milli-Q water. Prior to surface functionalization, any traces of water were removed by three times repeated rinsing and 15 min sonicating of the coverslip in acetone (VWR Chemicals). The coverslips were then immersed in 2 % (v/v) (3-Aminopropyl) triethoxysilane (Sigma-Aldrich) in acetone for 2 min followed by rinsing in Milli-Q water. The coverslips were then dried and baked for 30 min at 110 °C in a vacuum oven (Shel lab, USA). The flow cell was assembled from two silanized glass coverslips sealed together with melted parafilm stripes that formed a thin gap between them. The flow cell was further covalently functionalized with biotinylated polyethylene glycol (PEG-Biotin), providing an inert layer reducing the nonspecific interactions of the samples with glass. A PEG-Biotin (Biotin-PEG-SVA MW 5000, Laysan Bio Inc.) and PEG (mPEG-SVA MW 5000, Laysan Bio Inc.) were dissolved in 1 ml of 100 mM Sodium bicarbonate buffer (pH = 8.2) at 1:100 molar ratio (24 mM and 0.24 mM for PEG-Biotin and PEG, respectively). Then, 50 µl of the solution was applied into each flow cell followed by overnight incubation in a humid environment. After flushing the flow cell extensively with Tris buffer (1 mM, pH = 7.5), neutravidin (0.2 mg ml⁻¹) was introduced into the flow cells and incubated for 5 min. After a thorough wash of the flow cell with Tris buffer (1 mM, pH = 7.5), we introduced a 10-times-diluted solution of CTH (240 nM) and Cy3-50mer-ssDNA-biotin- (120 nM) mixture and incubated for 2 min. This procedure allowed tethering the complex to the neutravidin-coated glass surface for confocal imaging. After extensively flushing the flow cell with Tris buffer (1 mM, pH = 7.5) to removed unattached particles, 3 units of DNase I was introduced into the flow cell and incubated at 37 °C for 10 min. This step allowed to cleave any non-complexed Cy3-50mer-ssDNA-biotin attached to the surface of the flow cell. After additional

extensive flushing of the flow cell with Tris buffer (1 mM, pH = 7.5), the two open ends were fully sealed with silicone grease (Baysilone-Paste, GE Bayer Silocones) to protect the sample from drying during the experiment. The photon antibunching experiment was performed on a home-built setup based on Olympus IX71 confocal microscope.³⁴ A picosecond pulsed laser diode operated at 530 nm (PicoQuant, LDH-P-FA-530L) was used as an excitation source. The circularly polarized excitation light was obtained using a Berek compensator (Newport, Model 5540). The laser intensity was tuned by neutral density filters (Thorlabs, FW2AND). The excitation light was expanded by a beam expander to fill the back aperture of the objective lens before introducing into the microscope. The samples were excited through a high numerical aperture (NA) water immersion objective (UPlanSApo, 60 $\times$, NA 1.20, Olympus) at the excitation power of 0.85 W cm⁻². The raster scanning of the samples was achieved by moving the objective with a piezoelectric translation stage (Physik Instrumente, P-733.2CL,) controlled by a digital piezo controller (Physik Instrumente, E-710.4CL). Fluorescence from the samples was collected by the same objective, passed through a dichroic mirror (Semrock, Di02-R532) and separated from remaining excitation light by a long-pass emission filter (Semrock, BLP01-532R) after focusing on a 100 μ m pinhole (Thorlabs, P100S) by the tube lens of the microscope. Further, in the detection path, the Hanbury–Brown and Twiss type of photon correlation configuration was applied. The fluorescence photons of all wavelengths were divided by a 50/50 non-polarizing cube beam splitter (Thorlabs, CM1-BS013), and detected by two single-photon avalanche photodiodes (SPADs, PicoQuant, τ -SPAD-50) after passing through the set of identical bandpass filters (Semrock, FF01-612/69-25) mounted in front of each SPAD. The signals from the two SPADs were recorded by the TCSPC HydraHarp 400 module in the TTTR mode. SymPhoTime64 software (PicoQuant) was used for both the

acquisition of all the fluorescence microscopy data and analysis of the data. The number of emitters was determined by using the following expression:

$$\frac{N_C}{N_L} = 1 - \frac{1}{M} \quad \text{Equation 1}$$

where N_C is the intensity of the center peak at the delay time of 0 ns, N_L is the average intensity of lateral peaks and M is the number of emitters (see Figure S13). We recorded the photon coincidence data on a population of the particles that display relatively dark fluorescence signal, which enabled us to capture the data only on the sHD particles.

Calculations of the Number of ssDNA Molecules Associated with Histone Complexes. To calculate the total number of ssDNA molecules accommodated by one octamer (ssDNA_{oct}), we recall the equal DNA mass accommodation finding of ssOct and nucleosome.¹² For a typical octamer to accommodate ssDNA mass equal to dsDNA, two ssDNA molecules should wrap around one histone octamer such that the strands form loops between the binding sites along the spiral binding pathway. These loops are expected since the rise per base is different for ssDNA (0.7 nm/base) compared with dsDNA (0.34 nm/base pair). Therefore, the ssDNA length that can bind to the 14 binding sites was calculated as the length of a spiral spring (length=[(diameter× π ×number of turns)²+(pitch×number of turns)]^{0.5} = 52.51 nm). The total length of the ssDNA pieces associated with a histone octamer = 2 ssDNA molecules × 52.51 nm (helical path length around an octamer) × (0.7/0.34) = 216.22 nm. The total number of ssDNA molecules per one octamer (ssDNA^{oct}) could be then calculated by (ssDNA^{oct} = 216.22 nm / contour length of ssDNA molecule). The average number of 50mer-ssDNA molecules accommodated by 1 sHD in a cHD (ssDNA_{cHD}) can be calculated by solving equation 2:

$$ssDNA^{HD} = \frac{46 \times 6 \text{ molecules in an sHD particle} + 54 \times ssDNA^{cHD}}{100}$$

Equation 2

ASSOCIATED CONTENT

The authors declare no competing financial interests.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

List of Acronym; Native and SDS PAGE of individual histones and histones complexed with ssDNA; TEM, QELS, AFM and fluorescence quenching of histones complexed with ssDNA; table showing the calculated and experimentally obtained number of ssDNA in different histone-ssDNA complexes; and notes on the interaction of histone and ssDNA at different experimental conditions, the performance of TEM and gel electrophoresis at our experimental conditions and suggested pathway for ssDNA interaction at single nucleotide resolution. (PDF)

ACKNOWLEDGMENT

We thank Dr. R. Sougrat for the TEM and cryo-TEM images of histones and histone-DNA complexes. The authors would like to thank Prof. E. Yashima for providing the access to the dry-phase AFM facility in his lab. The authors would like thank Dr. M. Shuaib for the helpful discussions. Schematic illustrations in the figures were created by H. Hwang, scientific illustrator at King Abdullah University of Science and Technology. The research reported in this publication was supported by funding from King Abdullah University of Science and Technology (KAUST) and from Nagoya University.

AUTHOR INFORMATION

Corresponding Author

*Correspondence should be addressed to M.F.S. and S.H. (King Abdullah University of Science and Technology (KAUST), Biological and Environmental Sciences and Engineering Division, Thuwal 23955-6900, Saudi Arabia. E-mail: Maged.Serag@kaust.edu.sa, Satoshi.Habuchi@kaust.edu.sa).

Author Contributions

M.F.S initiated and guided the project in Nagoya University and developed the geometric model. M.F.S conceived the project with help from S.H in KAUST. M.F.S. and J.R.D. designed the fluorescence quenching, SDS-PAGE, and QELS experiments. M.F.S. and S.H. designed QELS, electron microscopy, and native PAGE experiments. H.P. and S.H. designed photon coincidence experiment. M.F.S conducted the TEM experiments, electrophoretic single particle tracking, dry-phase AFM, fluorescence quenching, and QELS experiments and analyzed the data. R.T. assisted the experiments in Nagoya University. A.A. conducted sucrose gradient, SDS-PAGE, native PAGE, QELS, and fluorescence measurements and analyzed the data. M.A. conducted the native gel experiments and the fluorescence quenching experiment in presence of salt. H.P. conducted the photon coincidence experiment and analyzed the data. N.K. advised the experimental work in Nagoya University. M.F.S. and S.H. wrote the manuscript with advice and key insights from J.R.D. Y.B. supervised the project in Nagoya University. S.H. supervised the project in KAUST. All authors discussed the results.

REFERENCES

1. Praetorius, F.; Dietz, H., Self-assembly of Genetically Encoded DNA-Protein Hybrid Nanoscale Shapes. *Science* **2017**, 1283, 1-8.
2. Zhou, K.; Ke, Y. G.; Wang, Q. B., Selective *In Situ* Assembly of Viral Protein onto DNA Origami. *J Am Chem Soc* **2018**, 140, 8074-8077.
3. Douglas, S. M.; Bachelet, I.; Church, G. M., A Logic-Gated Nanorobot for Targeted Transport of Molecular Payloads. *Science* **2012**, 335, 831-834.
4. Ke, Y. G.; Lindsay, S.; Chang, Y.; Liu, Y.; Yan, H., Self-Assembled Water-Soluble Nucleic Acid Probe Tiles for Label-Free RNA Hybridization Assays. *Science* **2008**, 319, 180-183.
5. Yamamoto, S.; De, D.; Hidaka, K.; Kim, K. K.; Endo, M.; Sugiyama, H., Single Molecule Visualization and Characterization of Sox2-Pax6 Complex Formation on a Regulatory DNA Element Using a DNA Origami Frame. *Nano Lett* **2014**, 14, 2286-2292.
6. Chandrasekaran, A. R.; Anderson, N.; Kizer, M.; Halvorsen, K.; Wang, X., Beyond the Fold: Emerging Biological Applications of DNA Origami. *Chembiochem* **2016**, 17, 1081-1089.
7. Lindman, B.; Dias, R., *DNA Interactions with Polymers and Surfactants*. John Wiley: Hoboken, N.J., 2008.
8. Smith, S.; Stillman, B., Stepwise Assembly of Chromatin During DNA Replication *In Vitro*. *EMBO J* **1991**, 10, 971-980.
9. Dyer, P. N.; Edayathumangalam, R. S.; White, C. L.; Bao, Y.; Chakravarthy, S.; Muthurajan, U. M.; Luger, K., Reconstitution of Nucleosome Core particles from Recombinant Histones and DNA. *Methods Enzymol* **2004**, 375, 23-44.

10. Laskey, R. A.; Honda, B. M.; Mills, A. D.; Finch, J. T., Nucleosomes Are Assembled by an Acidic Protein Which Binds Histones and Transfers Them to DNA. *Nature* **1978**, *275*, 416-420.
11. Adkins, N. L.; Swygert, S. G.; Kaur, P.; Niu, H.; Grigoryev, S. A.; Sung, P.; Wang, H.; Peterson, C. L., Nucleosome-Like, Single-stranded DNA (ssDNA)-Histone Octamer Complexes and the Implication for DNA Double Strand Break Repair. *J Biol Chem* **2017**, *292*, 5271-5281.
12. Palter, K. B.; Foe, V. E.; Alberts, B. M., Evidence for the Formation of Nucleosome-Like Histone Complexes on Single-Stranded DNA. *Cell* **1979**, *18*, 451-467.
13. Choi, J.; Majima, T., Conformational Changes of Non-B DNA. *Chem Soc Rev* **2011**, *40*, 5893-5909.
14. Caffarelli, E.; De Santis, P.; Leoni, L.; Savino, M.; Trotta, E., Interactions of the Histone Octamer with Single-Stranded DNA. Sedimentation Analysis and Low-Angle X-Ray Diffraction. *Biochim Biophys Acta* **1983**, *739*, 235-243.
15. Caffarelli, E.; Leoni, L.; Savino, M., Folding of Single-Stranded DNA on the Histone octamer. *FEBS Lett* **1985**, *181*, 69-73.
16. Fernandez-Busquets, X.; Daban, J. R., Histones Associated with Single-Stranded DNA Do Not Preclude the Formation of Double-Helical DNA. *Biochim Biophys Acta* **1995**, *1260*, 132-8.
17. Goel, T.; Mukherjee, T.; Rao, B. J.; Krishnamoorthy, G., Fluorescence Dynamics of Double- and Single-Stranded DNA Bound to Histone and Micellar Surfaces. *J Phys Chem B* **2010**, *114*, 8986-8993.
18. Workman, J. L.; Abmayr, S. M., *Fundamentals of Chromatin*. Springer: New York, 2014.

19. Anselmi, C.; Bocchinfuso, G.; De Santis, P.; Savino, M.; Scipioni, A., Dual Role of DNA Intrinsic Curvature and Flexibility in Determining Nucleosome Stability. *J Mol Biol* **1999**, *286*, 1293-1301.
20. Huang, R. C. C.; Bonner, J., Physical and Biological Properties of Soluble Nucleohistones. *J Mol Biol* **1964**, *8*, 54-64.
21. Mangenot, S.; Raspaud, E.; Tribet, C.; Belloni, L.; Livolant, F. Interactions Between Isolated Nucleosome Core Particles: A Tail-Bridging effect? *Eur Phys J E* **2002**, *7*, 221-231.
22. Gruber, H. J.; Hahn, C. D.; Kada, G.; Riener, C. K.; Harms, G. S.; Ahrer, W.; Dax, T. G.; Knaus, H. G., Anomalous Fluorescence Enhancement of Cy3 and Cy3.5 *Versus* Anomalous Fluorescence Loss of Cy5 and Cy7 Upon Covalent Linking to IgG and Noncovalent Binding to Avidin. *Bioconjugate Chem* **2000**, *11*, 696-704.
23. Li, S. E. Y., *Capillary Electrophoresis: Principles, Practice and Applications*. Elsevier: Amsterdam **1992**, *52*, pp 1-30.
24. Dalal, Y.; Wang, H.; Lindsay, S.; Henikoff, S., Tetrameric Structure of Centromeric Nucleosomes in Interphase Drosophila Cells. *Plos Biol* **2007**, *5*, 1798-1809.
25. Wang, H. D.; Dalal, Y.; Henikoff, S.; Lindsay, S., Single-Epitope Recognition Imaging of Native Chromatin. *Epigenet Chromatin* **2008**, *17*, 1-9.
26. Zlatanova, J.; Leuba, S. H., Stretching and Imaging Single DNA Molecules and Chromatin. *J Muscle Res Cell Motil* **2002**, *23*, 377-395.
27. Bolund, L. A.; Johns, E. W., The Selective Extraction of Histone Fractions From Deoxyribonucleoprotein. *Eur J Biochem* **1973**, *35*, 546-453.

28. Sugiyama, T.; Yuyama, K.; Masuhara, H., Laser Trapping Chemistry: From Polymer Assembly to Amino Acid Crystallization. *Acc Chem Res* **2012**, *45*, 1946-1954.
29. Fouad, M.; Jabasini, M.; Kaji, N.; Terasaka, K.; Tokeshi, M.; Mizukami, H.; Baba, Y., Microchip Analysis of Plant Glucosinolates. *Electrophoresis* **2008**, *29*, 2280-2287.
30. Serag, M. F.; Abadi, M.; Habuchi, S., Single-Molecule Diffusion and Conformational Dynamics by Spatial Integration of Temporal Fluctuations. *Nat Commun* **2014**, *5*, 5123.
31. Abadi, M.; Serag, M. F.; Habuchi, S., Single-Molecule Imaging Reveals Topology Dependent Mutual Relaxation of Polymer Chains. *Macromolecules* **2015**, *48*, 6263-6271.
32. Serag, M. F.; Habuchi, S., Conserved Linear Dynamics of Single-Molecule Brownian Motion. *Nat Commun* **2017**, *8*, 15675.
33. Gontard, L. C.; Ozkaya, D.; Dunin-Borkowski, R. E., A Simple Algorithm for Measuring Particle Size Distributions on an Uneven Background from TEM Images. *Ultramicroscopy* **2011**, *111*, 101-106.
34. Piwonski, H.; Michinobu, T.; Habuchi, S., Controlling Photophysical Properties of Ultrasmall Conjugated Polymer Nanoparticles Through Polymer Chain Packing. *Nat. Commun.* **2017**, *8*, 10.