Supplemental Information

Cryo-EM Structures of Centromeric Tri-nucleosomes Containing a Central CENP-A Nucleosome

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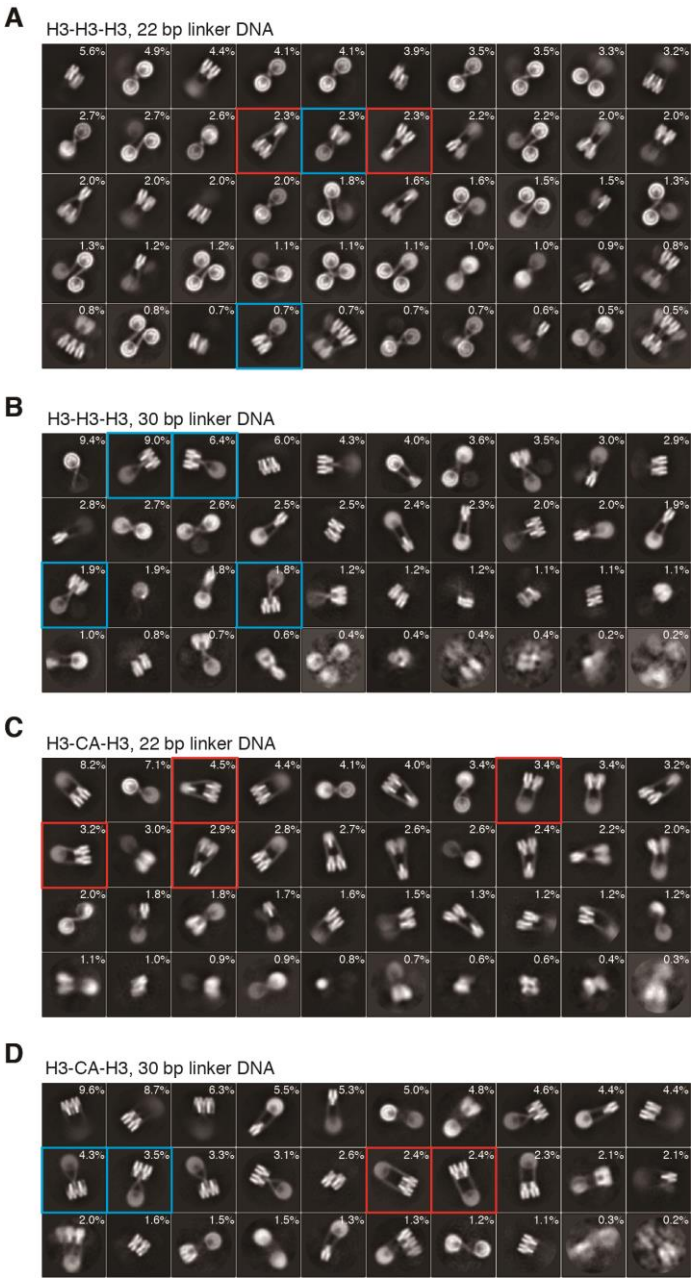

Figure S1. Particle distribution for Two-Dimensional Class Averages of the Tri-nucleosomes, related to Figure 3.

(A-D) 2D class averages of the H3-H3-H3 or H3-CA-H3 tri-nucleosomes containing 22 base-pair and 30 base-pair linker DNAs. Red and blue squares indicate the classes presented in Figure 3. Percentages of the particle distributions are shown in the boxes. Box size, 40 nm

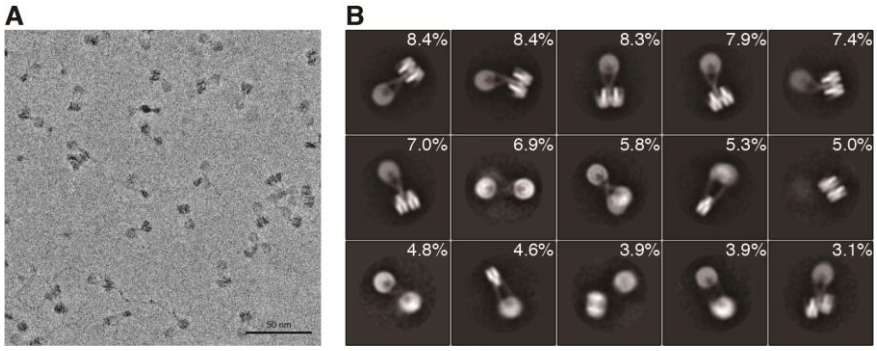

Figure S2. Phase Plate imaging and Two-Dimensional Class Averages of the CA-H3-CA Tri-nucleosomes, related to Figure 3.

(A) Raw digital micrograph of the CA-H3-CA tri-nucleosome containing 30 base-pair linker DNA were obtained with a Titan Krios cryo-electron microscope, using a Volta phase plate (VPP). Scale bar, 50 nm. (B) Representative 2D class averages of the CA-H3-CA tri-nucleosomes containing 30 base-pair linker DNA. Box size, 53 nm.

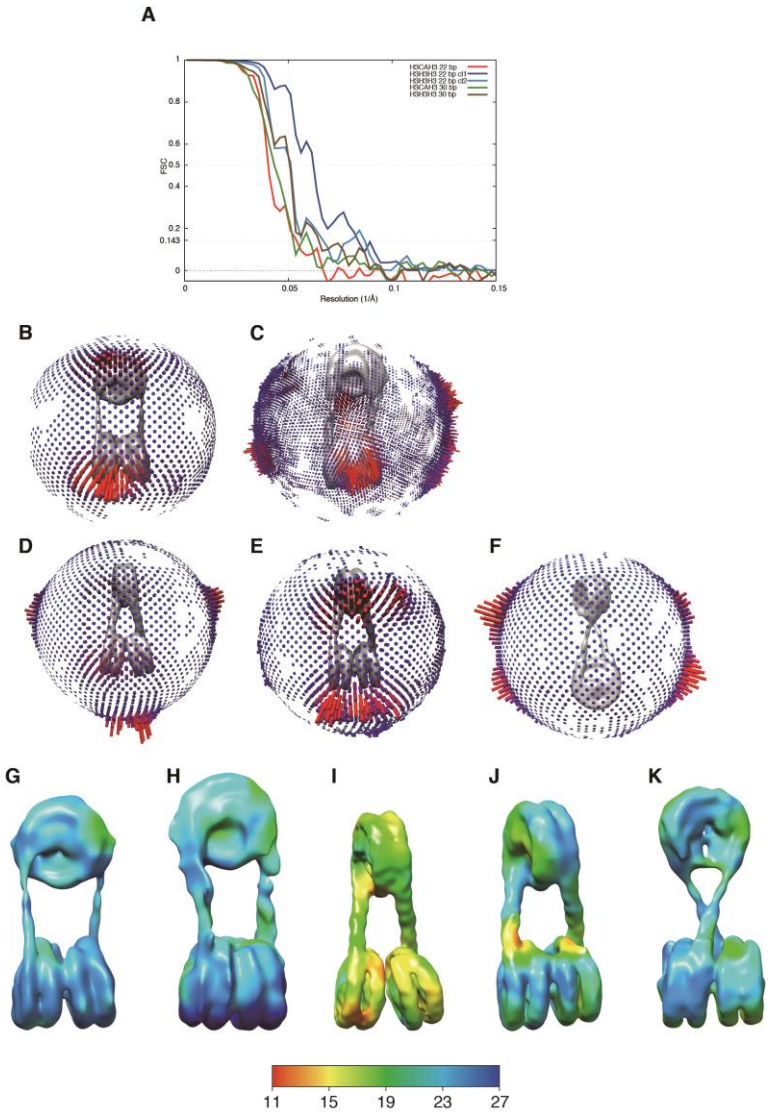

Figure S3. Cryo-EM Analysis of the Tri-nucleosomes, related to Figure 4 and 5. (A) The Fourier shell correlations between independently refined particle image datasets indicate spatial resolutions of 12.3 Å (Figure 4A), 15.1 Å (Figure S2), 18.7 Å (Figure 4B), 19.6 Å (Figure 5A), and 15.7 Å (Figure 5B) for H3-H3-H3 with the 22 base-pair linker DNA (class 1), H3-H3-H3 with the 22 base-pair linker DNA (class 2), H3-CA-H3 with the 22 base-pair linker DNA, H3-CA-H3 with the 30 base-pair linker DNA, and H3-H3-H3 with the 30 base-pair linker DNA, respectively (FSC=0.143). (B-F) 3D-Euler angle distributions of all particles contributing to the final 3D reconstructions of the tri-nucleosomes in Figure 4B, Figure 5A, Figure 4A, Figure S2, and Figure 5B. (G-K) Local resolution maps of the tri-nucleosomes in Figure 4B, Figure 5A, Figure 4A, Figure S2, and Figure 5B.

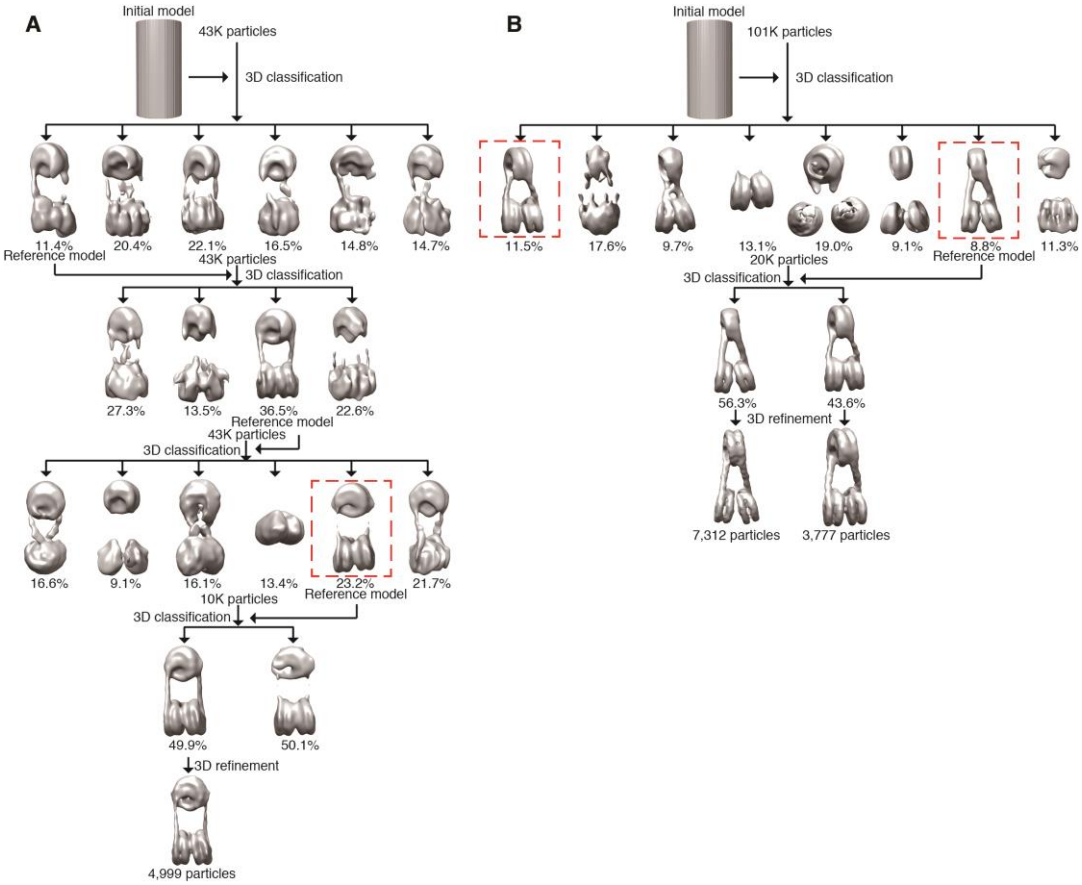

Figure S4. Workflow for Cryo-EM Processing of the Trinucleosomes containing 22 base-pair linker DNAs, related to Figure 4.

(A and B) 3D classifications of the H3-CA-H3 (A) and the H3-H3-H3 (B) tri-nucleosomes containing 22 base-pair linker DNAs. Boxes with red dashed lines indicate the classes selected for the next round of 3D classification.

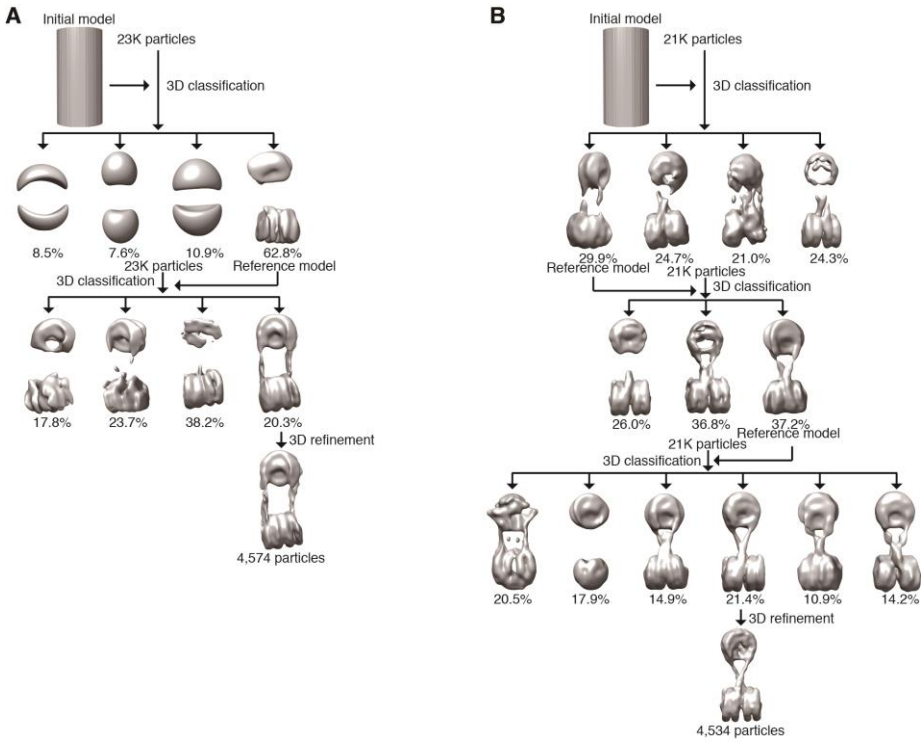

Figure S5. Workflow for Cryo-EM Processing of the Trinucleosomes containing 30 base-pair linker DNAs, related to Figure 5.

(A and B) 3D classifications of the H3-CA-H3 (A) and the H3-H3-H3 (B) tri-nucleosomes containing 30 base-pair linker DNAs.

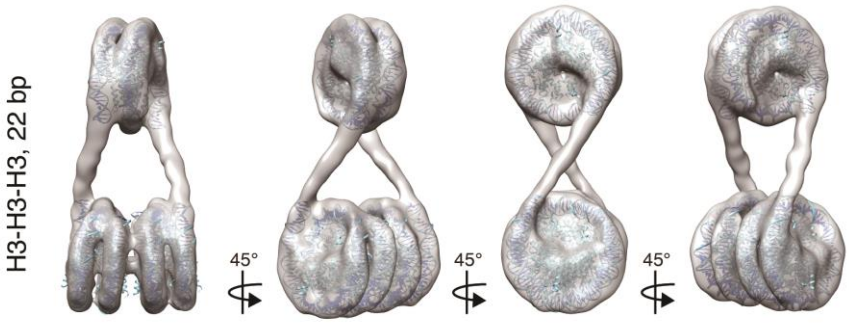

Figure S6. Three-dimensional Structure of the H3-H3-H3 Tri-nucleosome containing the 22 base-pair Linker DNA (class 2), related to Figure 4.

Semi-transparent iso-surface representation of the reconstructed electron potential of the H3-H3-H3 tri-nucleosome (class 2), contoured at 3.7 sigma above mean density. A model of the crystal structure of the H3 nucleosome (PDB: 3LZ0) was placed in the cryo-EM map.

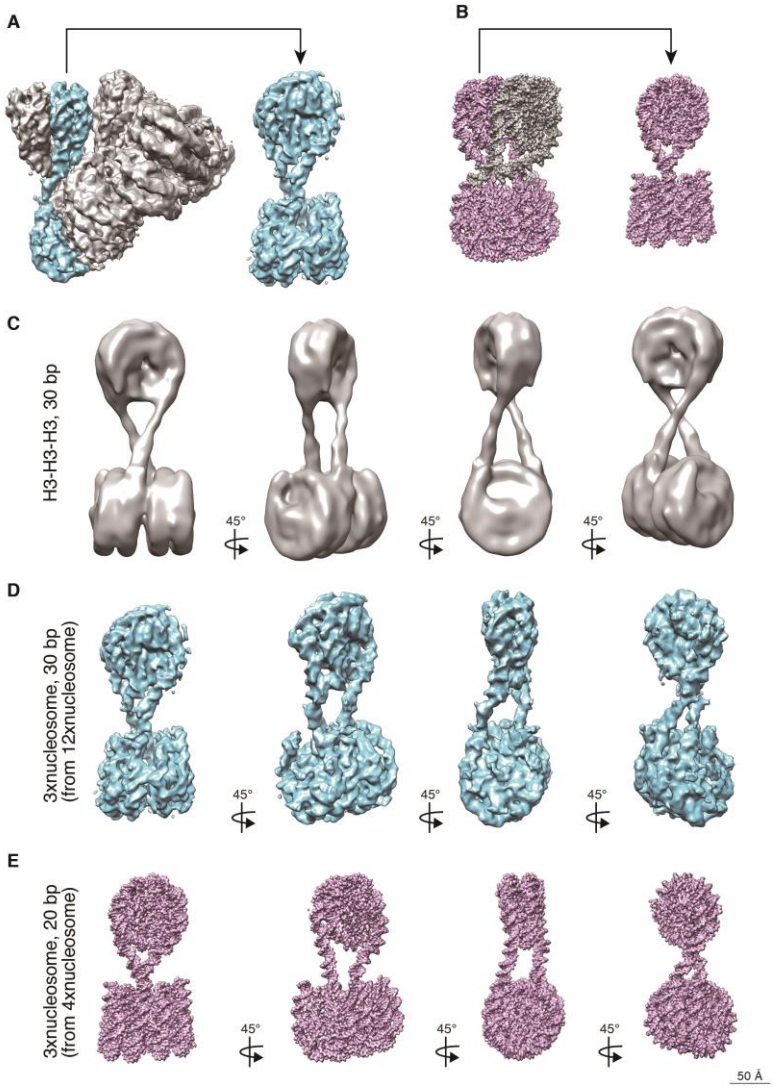

Figure S7. Structural Comparison of the H3-H3-H3 Tri-nucleosome with the 30 Base-pair Linker DNAs, the Corresponding Tri-nucleosome within the Poly-nucleosome 12-mer (EMD-2600), and the Corresponding Tri-nucleosome within the tetra-nucleosome (PDB: 1ZBB) related to Figure 5.

(A) A tri-nucleosome (light blue) segment extracted from the poly-nucleosome 12-mer structure (Song et al., 2014). (B) A tri-nucleosome (pink) segment extracted from the tetra-nucleosome structure (Schalch et al., 2005). (C) Cryo-EM structure of the H3-H3-H3 tri-nucleosome with the 30 base-pair linker DNA, corresponding to Figure 5B. (D) Cryo-EM structure of a tri-nucleosome segmented from the poly-nucleosome 12-mer structure (light blue). (E) Cryo-EM structure of a tri-nucleosome segmented from the tetra-nucleosome structure (pink). Scale bar, 50 Å.