



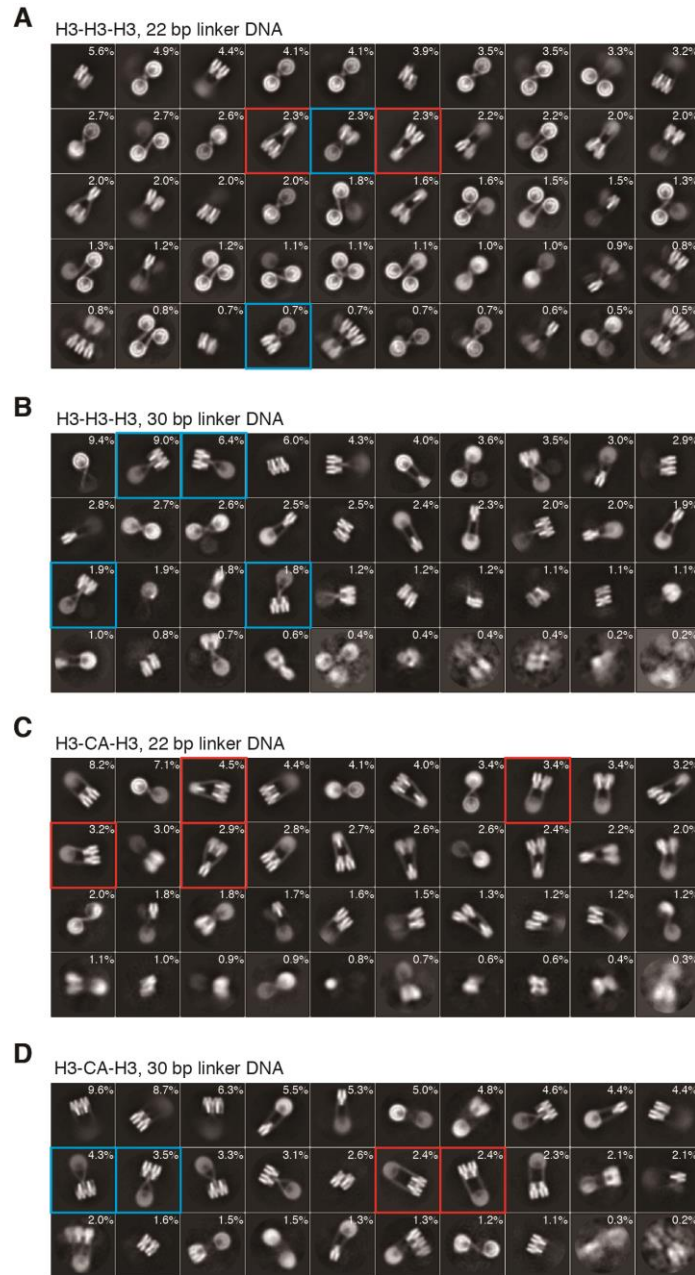
# Cryo-EM Structures of Centromeric Tri-nucleosomes Containing a Central CENP-A Nucleosome

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## **Supplemental Information**

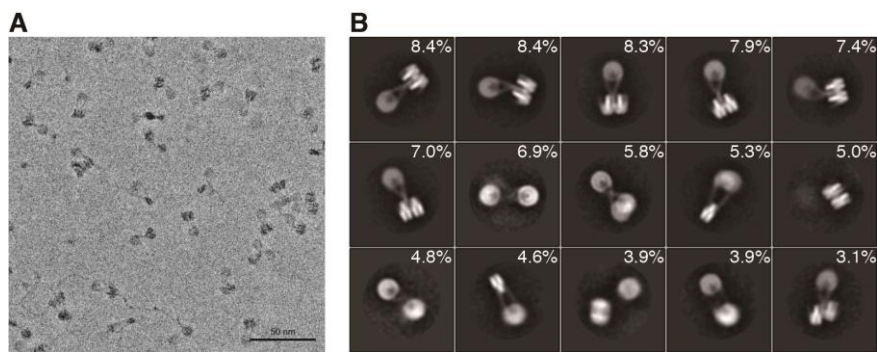
### **Cryo-EM Structures of Centromeric Tri-nucleosomes Containing a Central CENP-A Nucleosome**

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Wataru Kobayashi, Midori Suzuki, Yasuhiro Arimura, Tetsuya Hori, Tatsuo  
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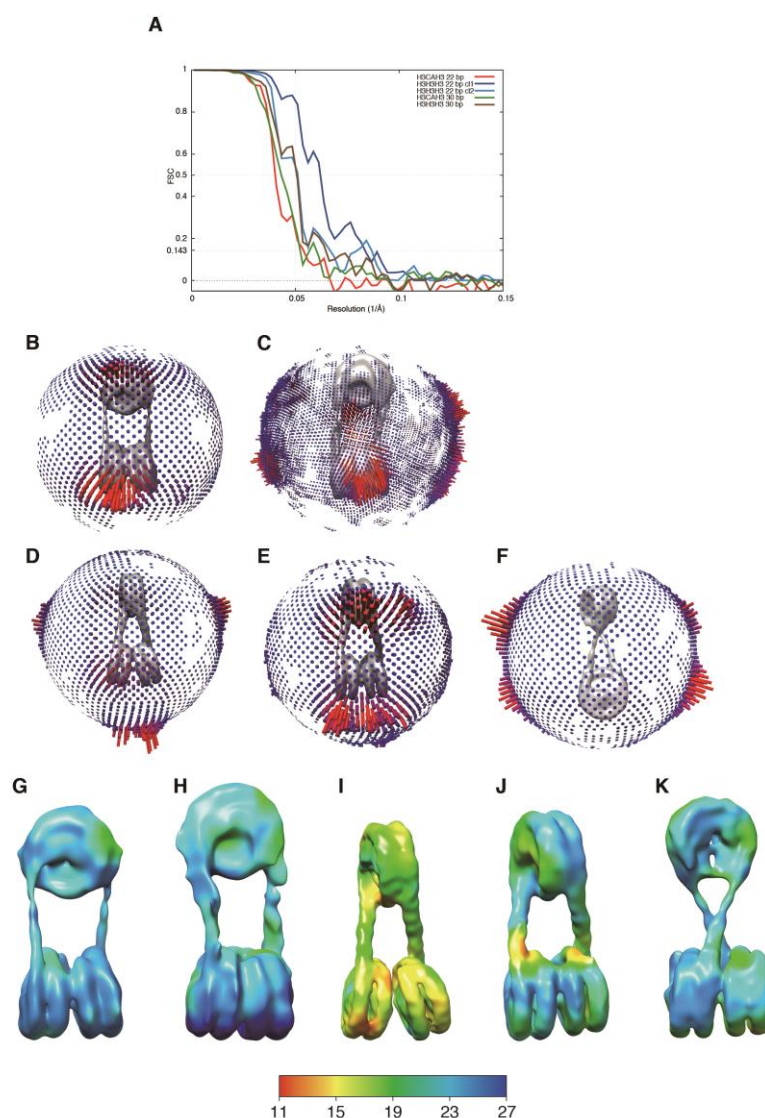
**Figure S1. Particle distribution for Two-Dimensional Class Averages of the Tri-nucleosomes, related to Figure 3.**

(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



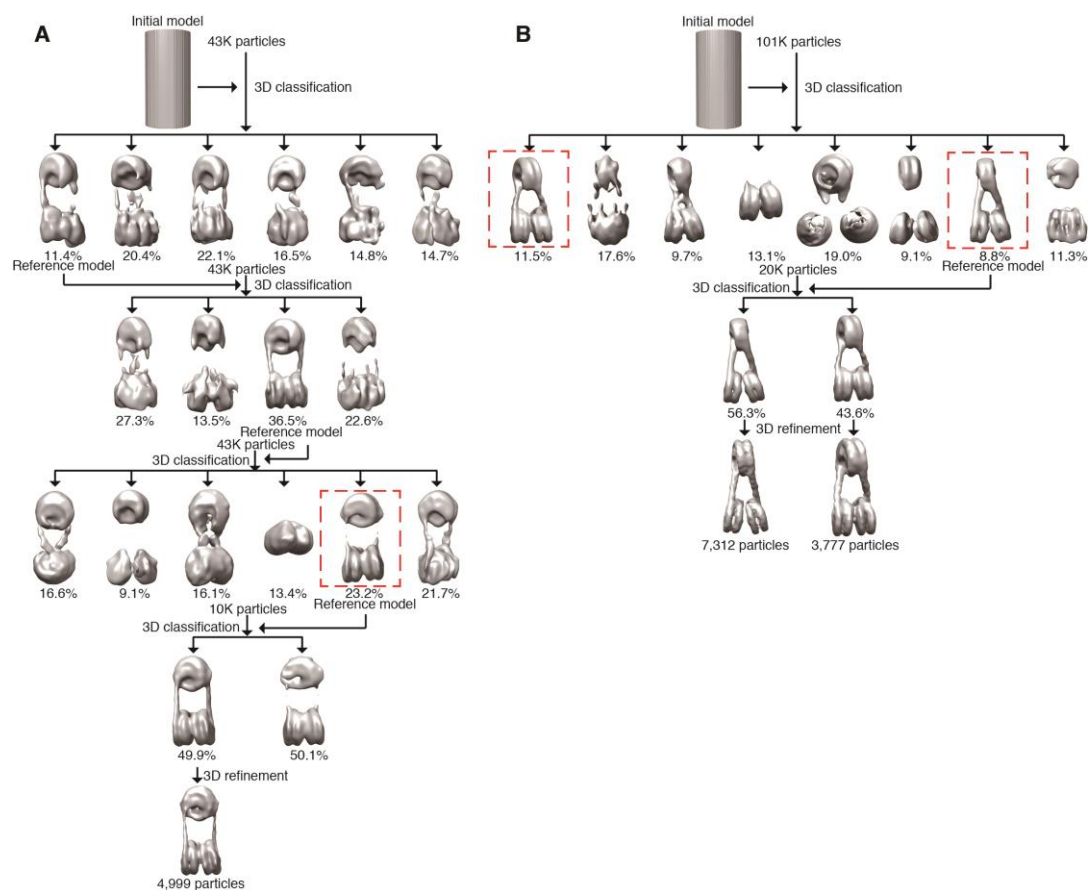
**Figure S2. Phase Plate imaging and Two-Dimensional Class Averages of the CA-H3-CA Tri-nucleosomes, related to Figure 3.**

(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.



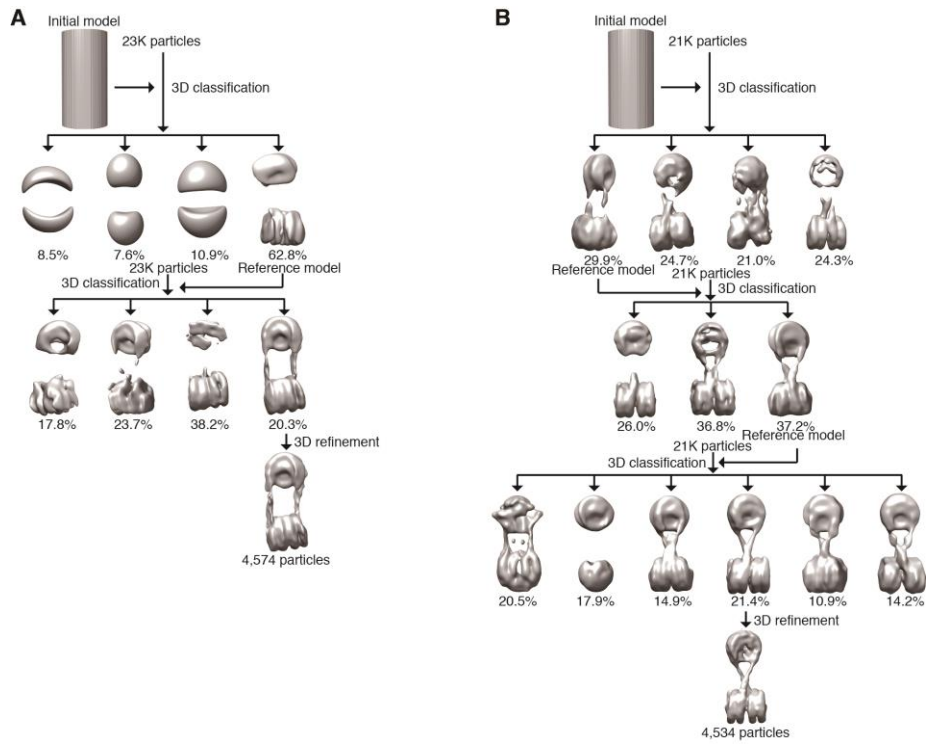
**Figure S3. Cryo-EM Analysis of the Tri-nucleosomes, related to Figure 4 and 5.**

(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.



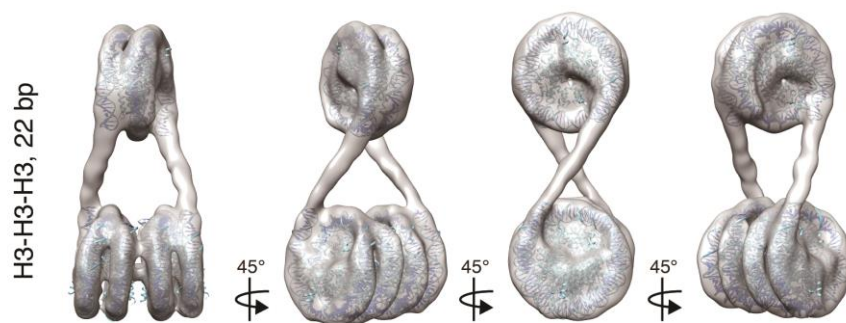
**Figure S4. Workflow for Cryo-EM Processing of the Trinucleosomes containing 22 base-pair linker DNAs, related to Figure 4.**

(A and B) 3D classifications of the H3-CA-H3 (A) and the H3-H3-H3 (B) trinucleosomes 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.**

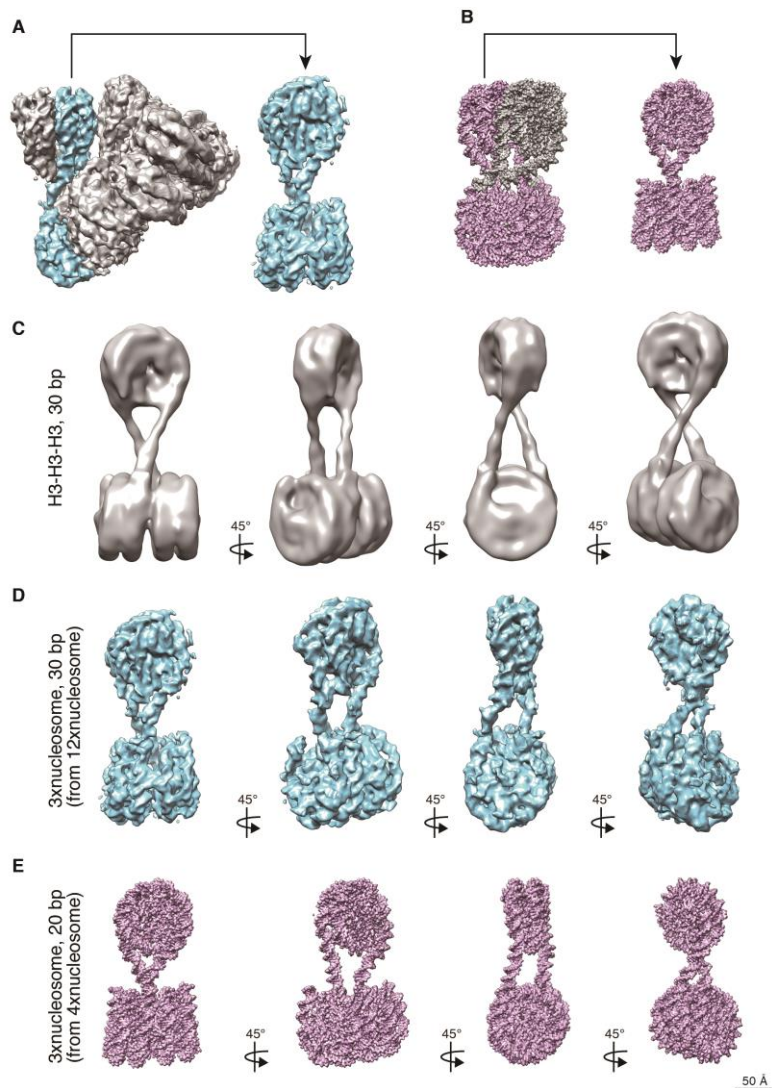
(A and B) 3D classifications of the H3-CA-H3 (A) and the H3-H3-H3 (B) trinucleosomes containing 30 base-pair linker DNAs.



**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.