Supplementary Material

Cryo-EM of NCP interactions in trans

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Figure S1: Nucleosome core particle dimers

(A) Chromatogram and SDS-PAGE showing assembly of the native octamer. Excess of H2A/H2B dimer, used in the octamer assembly, was removed by size exclusion chromatography. Black line shows migration of the octamer. Red line shows migration of the H2A/H2B dimer. Selected fractions, marked with a black bar on the chromatogram, were analyzed by SDS-PAGE.

(B) Native gel showing NCP assembly.

(C) Representative 2D class averages showing pairs of nucleosome core particles in many different orientations. Many details are visible in 2D class averages.
Supplementary Figure 2
**Figure S2: Cryo-EM of NCP dimer particles**

**A** Cryo-EM map and local resolution estimation of the NCP at 4.7 Å (0.143 cutoff in FSC curve).

**B** Fourier shell correlation (FSC) curve showing the resolution of cryo-EM map of the NCP.

**C** Cryo-EM maps of NCP 1 of the 3 classes that have the adjacent NCP 2.

**D** A lower counter of the 3 classes that have the adjacent NCP 2. The density for NCP 2 is shown in red. NCP 1 is shown in blue.
Figure S3: Local resolution calculation of NCP dimer reconstructions

Local resolution estimate determined by Relion. NCP 1 is resolved at 8-10 Å, while the NCP 2 is present at much lower resolution (15-25 Å). The side view is shown at higher contour level to show local resolution of NCP 1.
Figure S4: NCP pairs can adopt multiple conformations

(A) Lower contour level of cryo-EM maps A4 and A5 showing the contact NCP 1 and NCP 2. The NCP 1 is shown in light blue and the NCP 2 is shown in red.
Figure S5: Fitting of the X-ray model into cryo-EM maps.

The X-ray model of the NCP (PDB ID 3LZ1) was fitted into the Classes A1-A5. Cryo-EM density for both NCPs is shown in transparent blue. The molecular model for NCP 1 is shown in blue and for NCP 2 in red.
Supplementary Figure 6

A. 30nm fiber tetra-nucleosome

B. Diagram showing acidic patch, dyad, and H3 tail.

C. Diagram showing H4 tail and H4 N25.

D. Graph showing FSC against resolution in Å.

E. Comparison of NCP side contacting 2nd NCP and NCP side without the 2nd NCP.
Figure S6: H4-tail delocalizes when adjacent to the second nucleosome core particle

(A) Comparison of the NCP models fitted into the tetra-nucleosome structure of 30 nm fiber (Song et al, 2014). In the tetra-nucleosome, adjacent NCPs are stacked with histone octamers facing each others.

(B) NCP model with depicted histones, histone tails and acidic patch (red circle).

(C) Cryo-EM map of the combined reconstruction of the classes A1-A3 at 8.3 Å (0.143 cutoff in FSC curve).

(D) Fourier shell correlation (FSC) curve showing the resolution of cryo-EM map of the NCP.

(E) H4 tail is changing the conformation upon the interactions with the second NCP.

References: