

## **Supplementary Material**

Cryo-EM of NCP interactions in trans

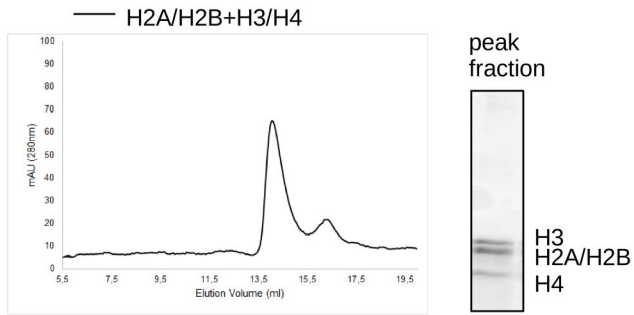
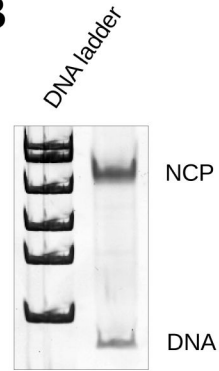
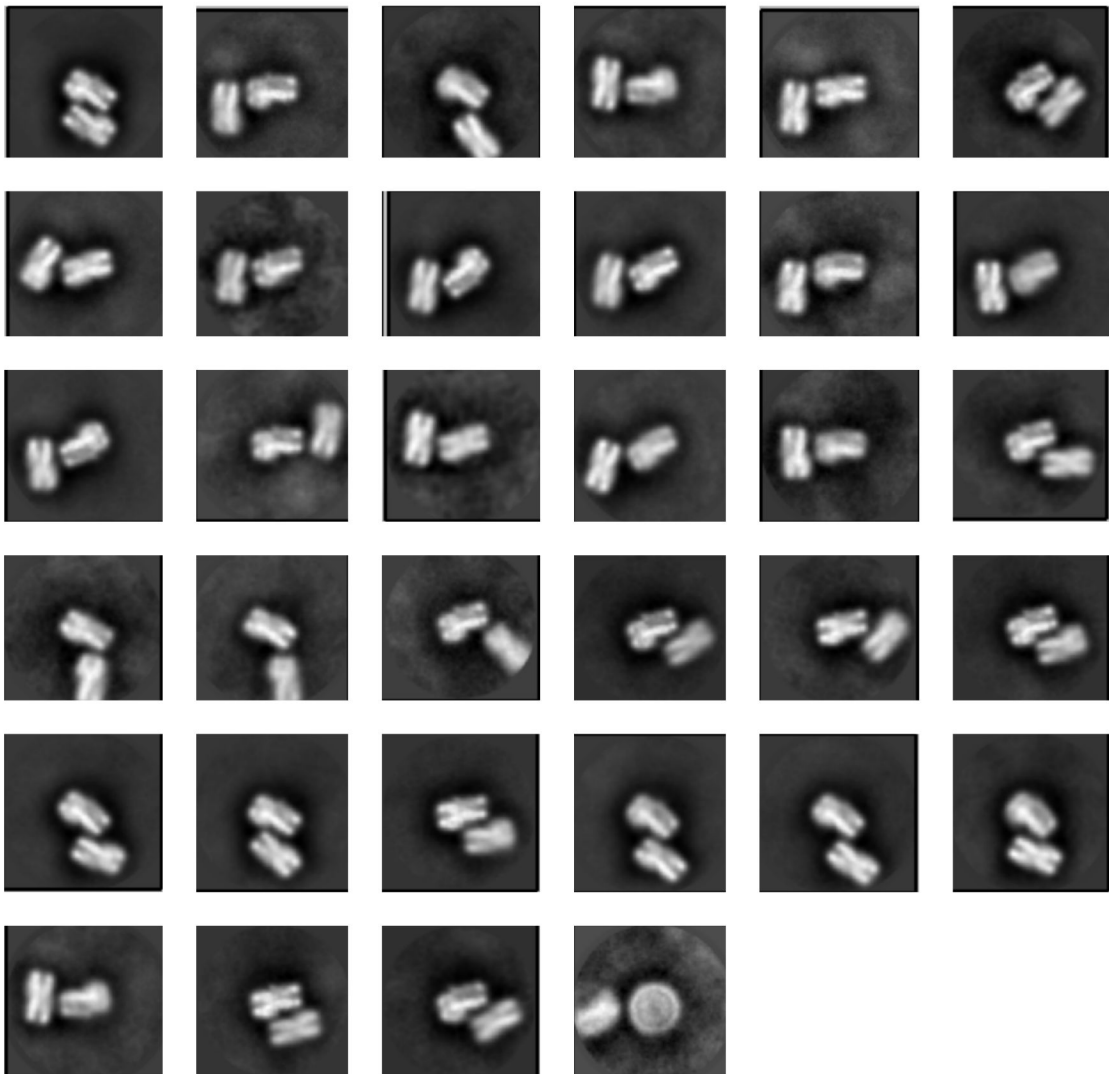
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**A****B****C**

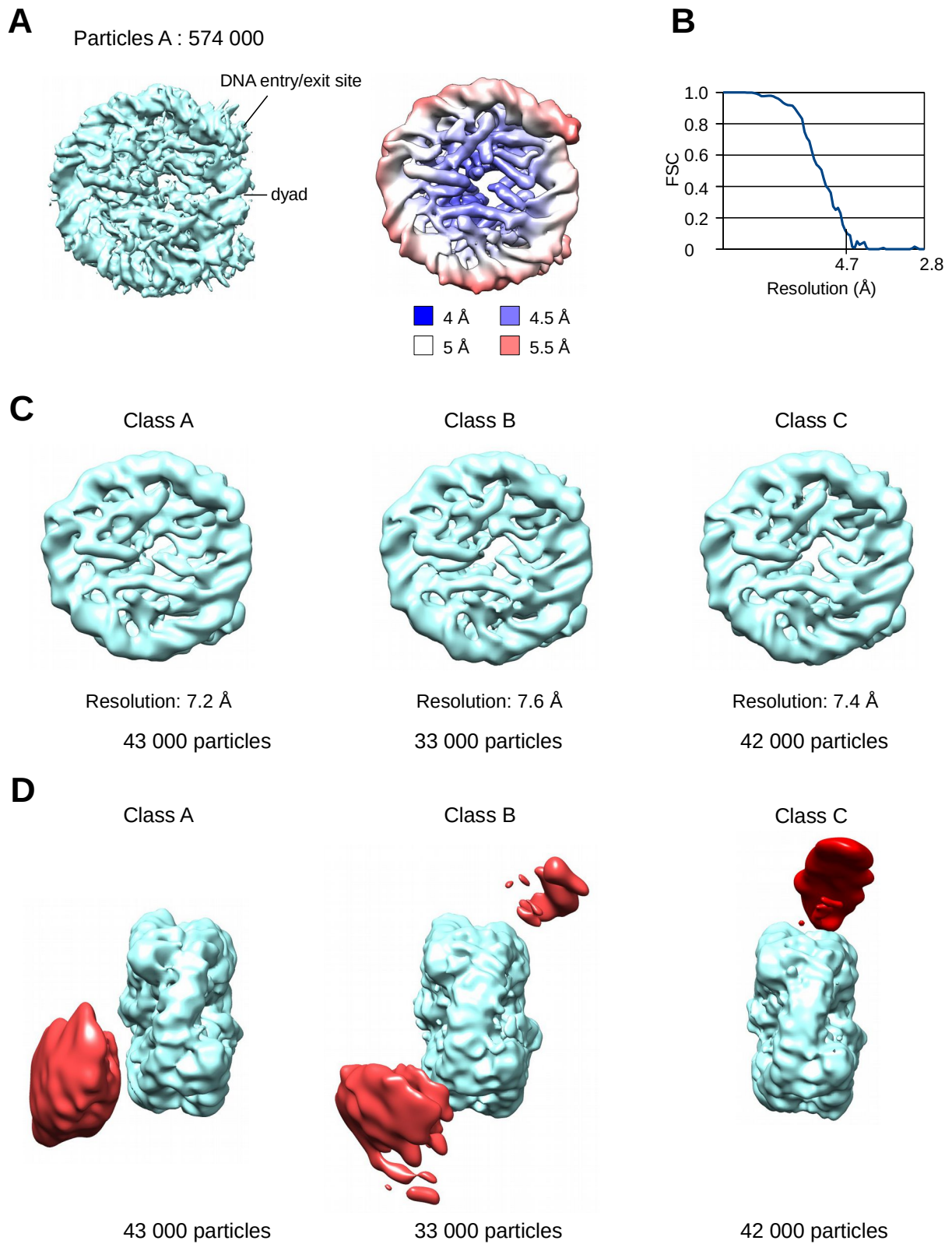
Supplementary Figure 1

### **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**

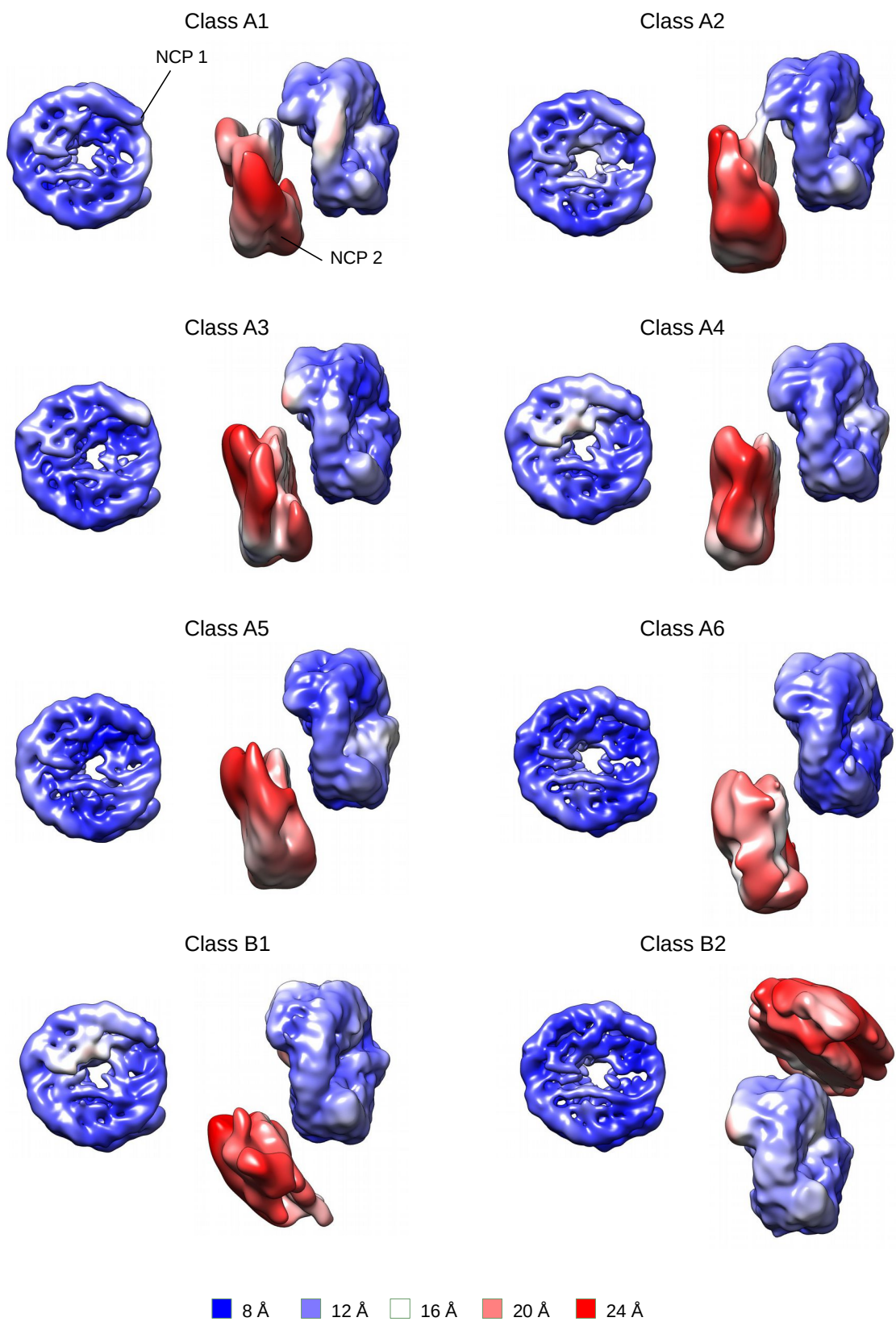
**Figure S2: Cryo-EM of NCP dimer particles**

**(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.

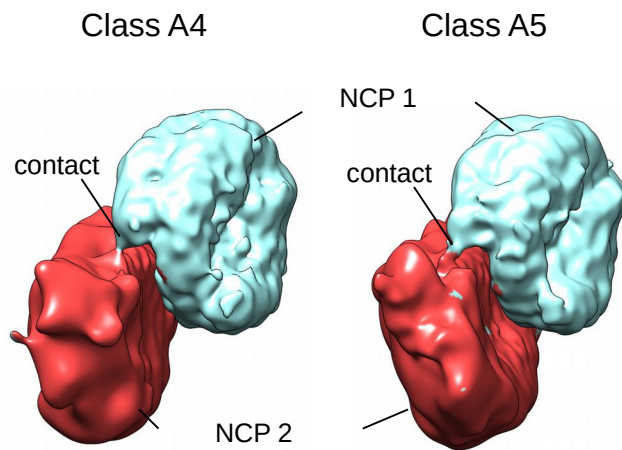
**(D)** A lower counter of the 3 classes that have the adjacent NCP 2. The density for NCP 2 is shown in red. NCP 1 is shown in blue.



**Supplementary Figure 3**

**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.

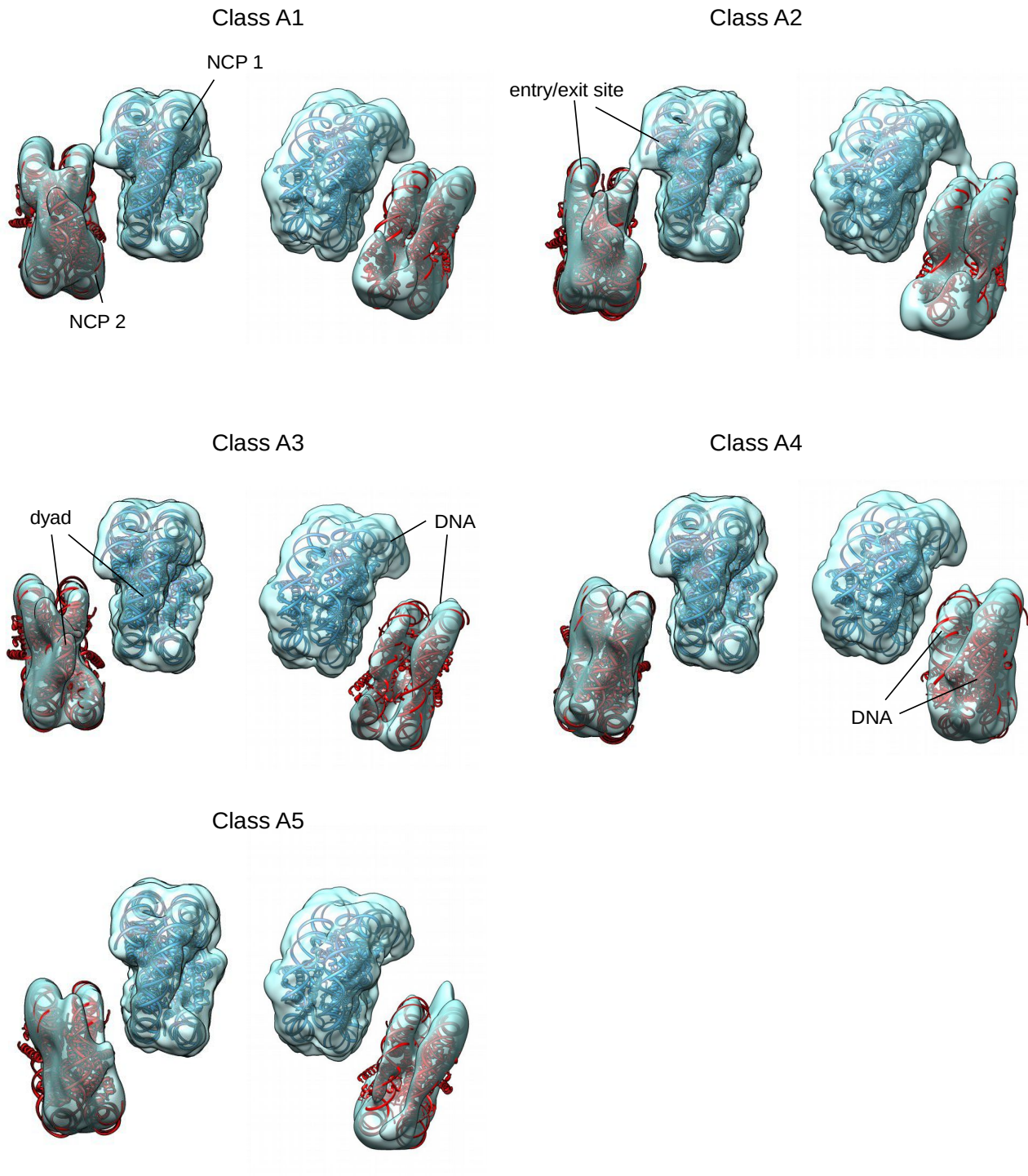


## Supplementary Figure 4

### 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.

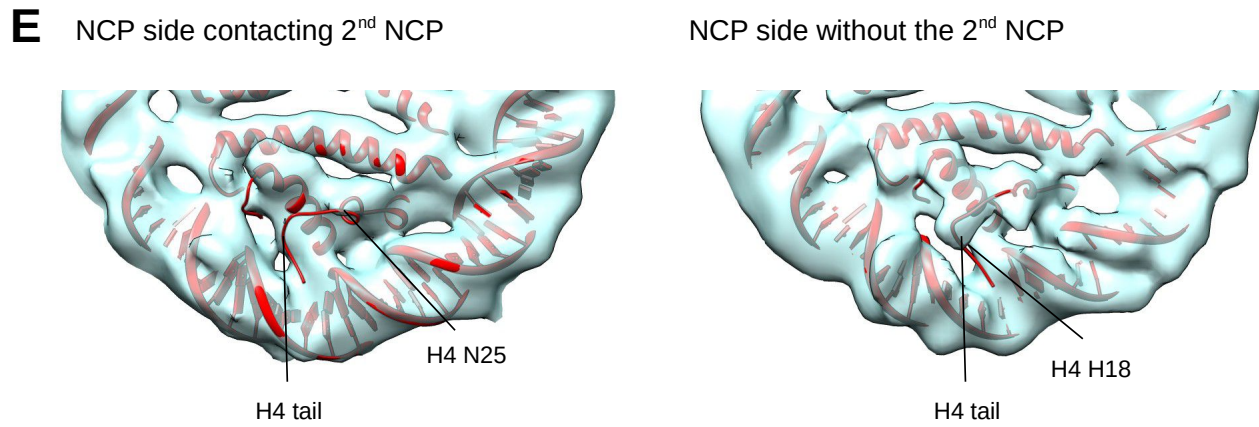
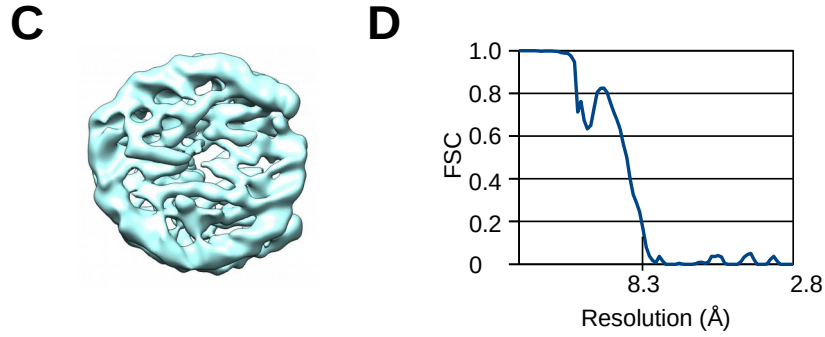
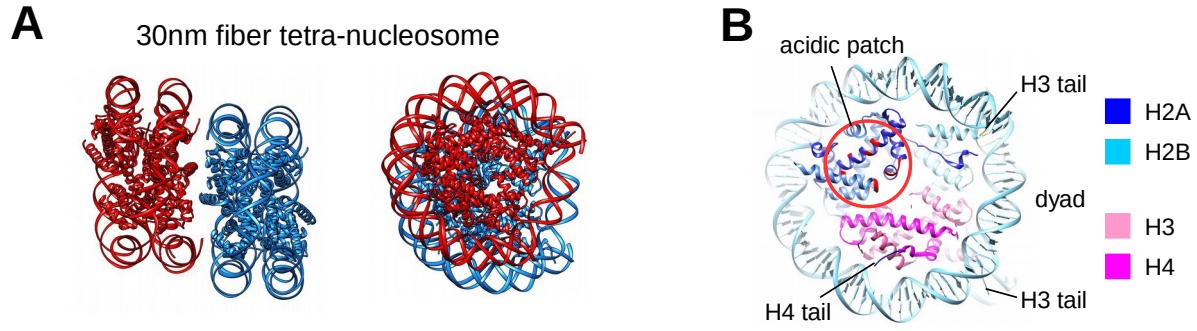




**Supplementary Figure 5**

**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**

**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 :**

Song F, Chen P, Sun D, Wang M, Dong L, Liang D, Xu R-M, Zhu P & Li G (2014) Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units. *Science* 344: 376–380