Appendix

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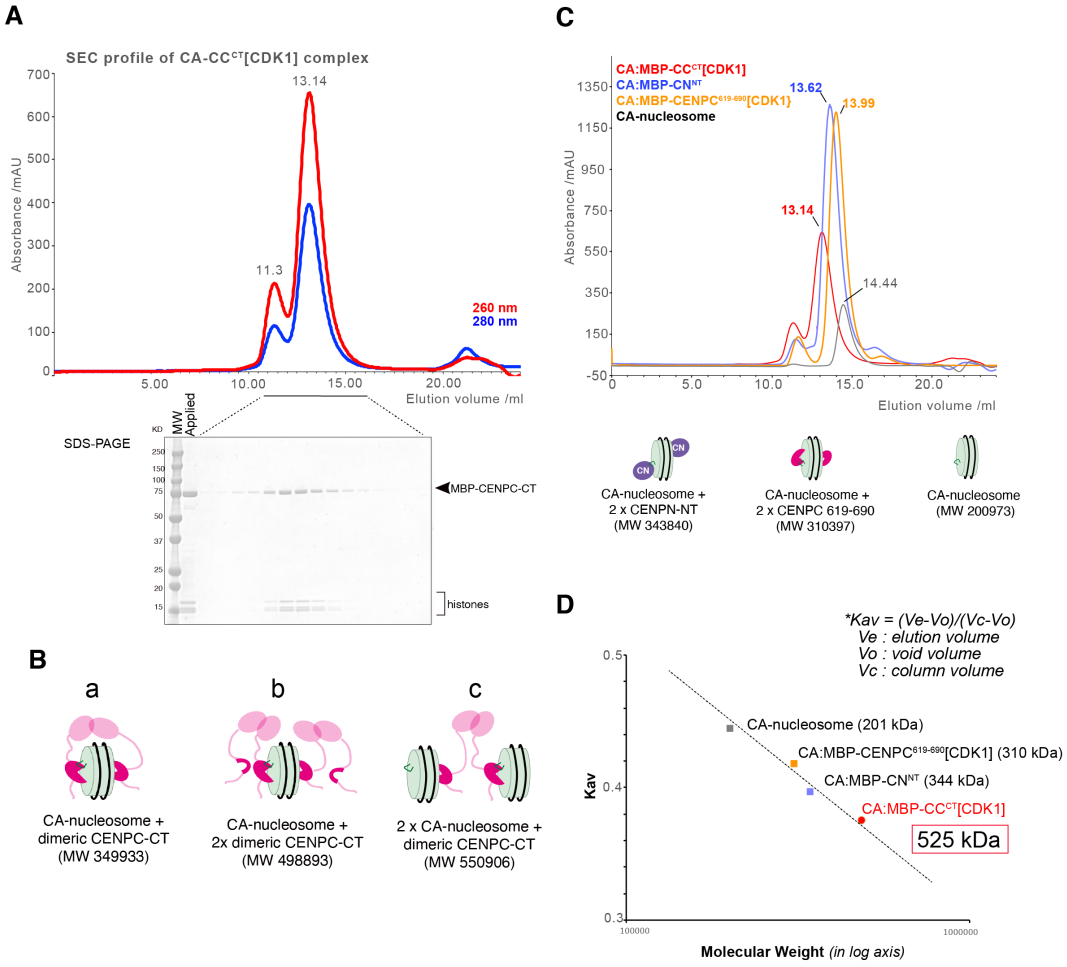
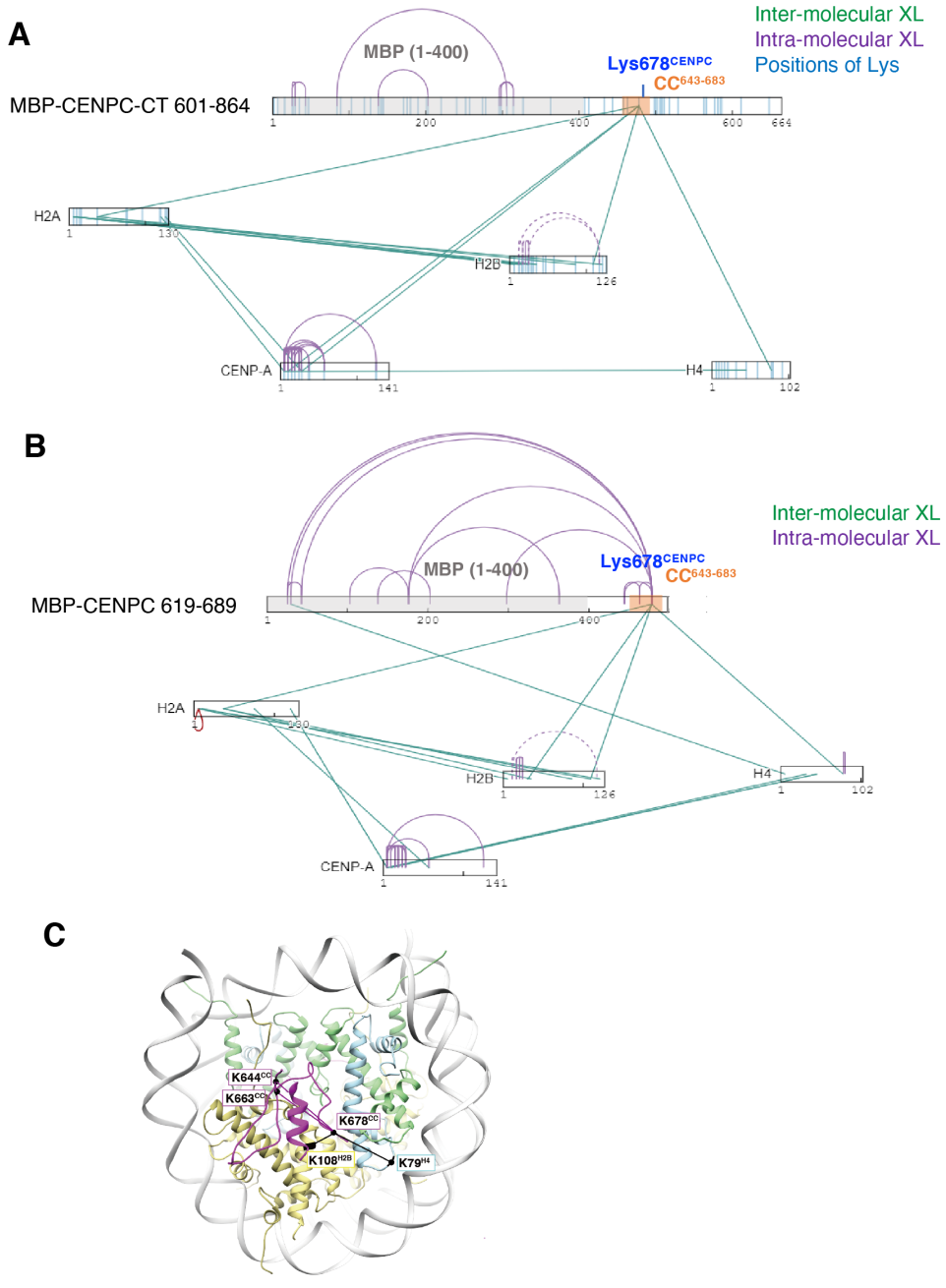


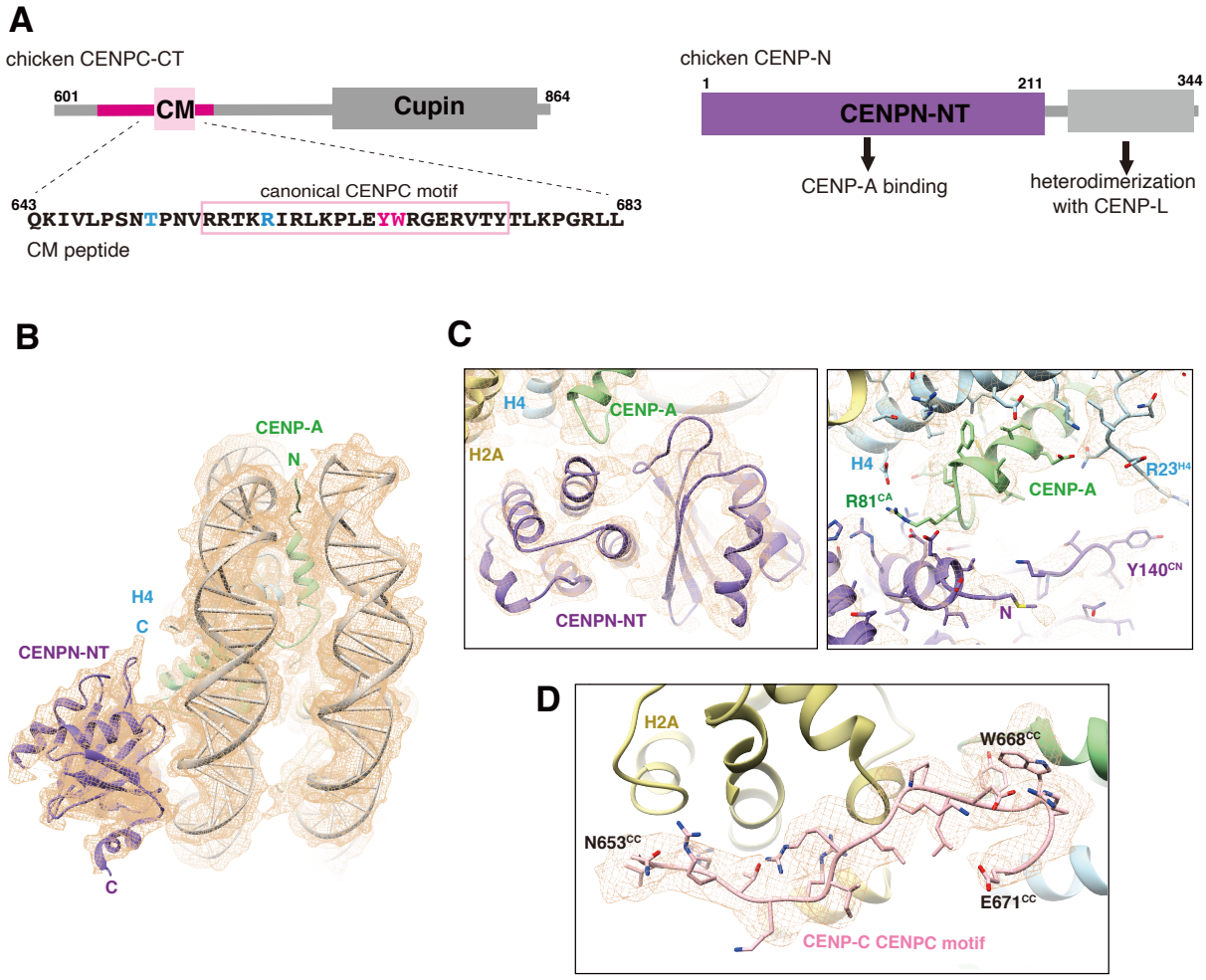
Appendix Figure S1 - Size exclusion chromatography analysis of the CENP-A nucleosome complexed with CENPC-CT

A) Size exclusion chromatography (SEC) profiles of the CA-CC^{CT} complex obtained using Superose 6 10/30 column. The elution of the complex was monitored using absorbance at 280 and 260 nm. The complex was prepared by mixing the CENP-A nucleosome and phosphorylated MBP-CENPC-CT (CC^{CT}[CDK1]). The fractions indicated by a black line were analyzed by SDS-PAGE. The phosphorylated MBP-CENPC-CT and histones in the CENP-A nucleosome were co-eluted in the peak fractions (elution volume, 13.14 ml). **B)** Models of the stoichiometric CA-CC^{CT} complex. The theoretical molecular weight of each model, including an MBP tag, is indicated. **C)** Comparison of SEC profiles (absorbance at 260 nm) of the CENP-A nucleosome complexes: CA-MBP-CC^{CT}[CDK1], the complex with phosphorylated MBP-CENPC-CT; CA-MBP-CN^{NT}, the complex with MBP-CENPN-NT; CA-MBP-CENPC$^{619-689}$[CDK1], the complex with phosphorylated CENP-C fragment (aa 619-689) which does not contain the dimeric region. The profile of the CENP-A nucleosome (CA-nucleosome) is also presented. The elution volume of each complex or nucleosomes is indicated in the profile. The molecular weight of the CENP-A nucleosome and the complexes are indicated below. **D)** Calibration curve obtained with CA-MBP-CN^{NT}, CA-MBP-CENPC$^{619-689}$[CDK1] and the CENP-A-nucleosome as standard proteins. The molecular weight of the CA-CC^{CT}[CDK1] complex is estimated to be 525 kDa. The model "a" shown in (B), in which the dimeric CENPC-CT symmetrically binds to both faces of CENP-A the nucleosome using two monomers, is less likely to occur in solutions.



Appendix Figure S2 - Crosslinking mass spectroscopy (XL-MS) analysis of the CA-CC^{CT} complex

A) XL-MS interactions depicted in relation to MBP-fused CENPC-CT fragment (phosphorylated CENP-C, aa 601-864) and histones, including CENP-A. Color bars represent protein sequences. Black and purple lines show inter- and intra-protein links, respectively. **B)** XL-MS interactions depicted in relation to MBP-fused CENPC$^{601-689}$ fragment (phosphorylated CENP-C, aa 619-689) and histones, including CENP-A. **C)** The cross-linked sites in CENPC-CT observed in (A) and (B) are shown on the cryo-EM structure. Black lines show the links between CENP-C and histones. The cross-linked sites within CENP-C (purple lines), in which K678 was linked with K644 and K633, were observed in the CENP-A nucleosome complexed with MBP-CENPC$^{601-689}$ fragment.



Appendix Figure S3 - Cryo-EM structure of the CA-CC^{pep}-CN^{NT} complex
A) Sequence of the chicken CENPC motif peptide (CM peptide) and the chicken CENPN-NT used for cryo-EM single particle image analysis. **B)** Cryo-EM density map of the CA-CC^{pep}-CN^{NT} complex at 4.2 Å resolution. The map is shown in a mesh representation with the final structural model depicted in the ribbon representation. **C)** The structure of the interface between CENP-N and CENP-A. R81 in the RG loop^{CENP-A} is recognized by CENPN-NT, as shown in the right panel. The histone H4 residue, R23^{H4}, likely contacts Y140 of CENP-N (right panel). **D)** Cryo-EM density map for the CM peptide bound to the CENP-A nucleosome. The density of both terminal parts of the CENPC motif peptide was not observed