Supplementary Information

DNA-bridging by an archaeal histone variant via a unique tetramerisation interface

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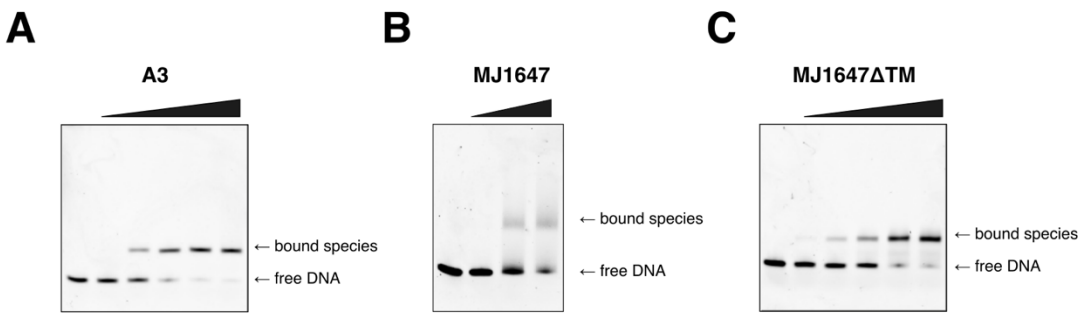
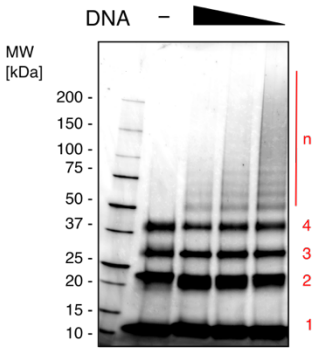


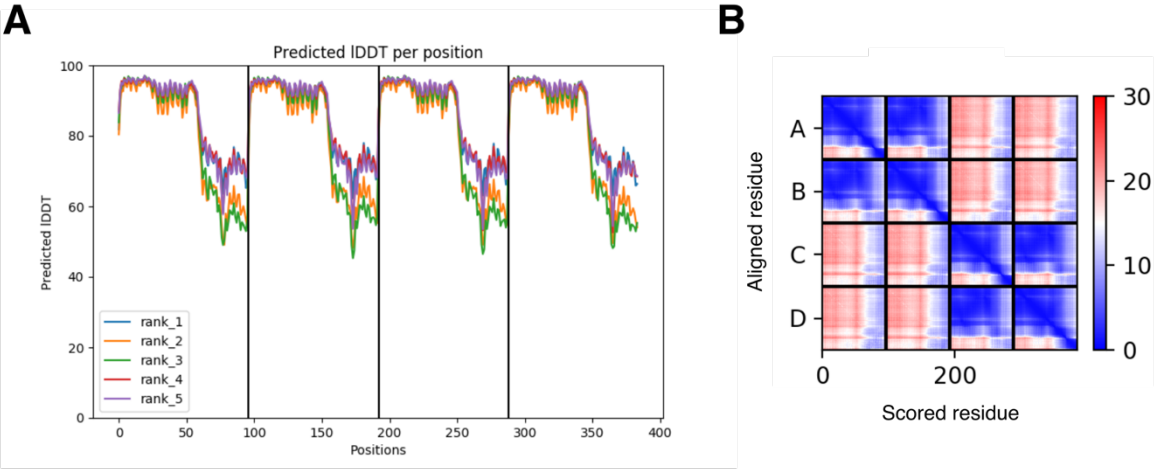
Supplementary Figure S1

EMSA experiments testing binding to 30 bp DNA templates. Increasing concentrations of histones A3 (A), MJ1647 (B) and the deletion variant MJ1647□TM (C) were incubated with the respective 32P-labelled DNA templates and resolved on native polyacrylamide gels. Protein concentrations were 2, 4, 6, 12, 14 μM histone monomers for A3 and MJ1647DTM, 4, 8, 12 μM for MJ1647.



Supplementary Figure S2

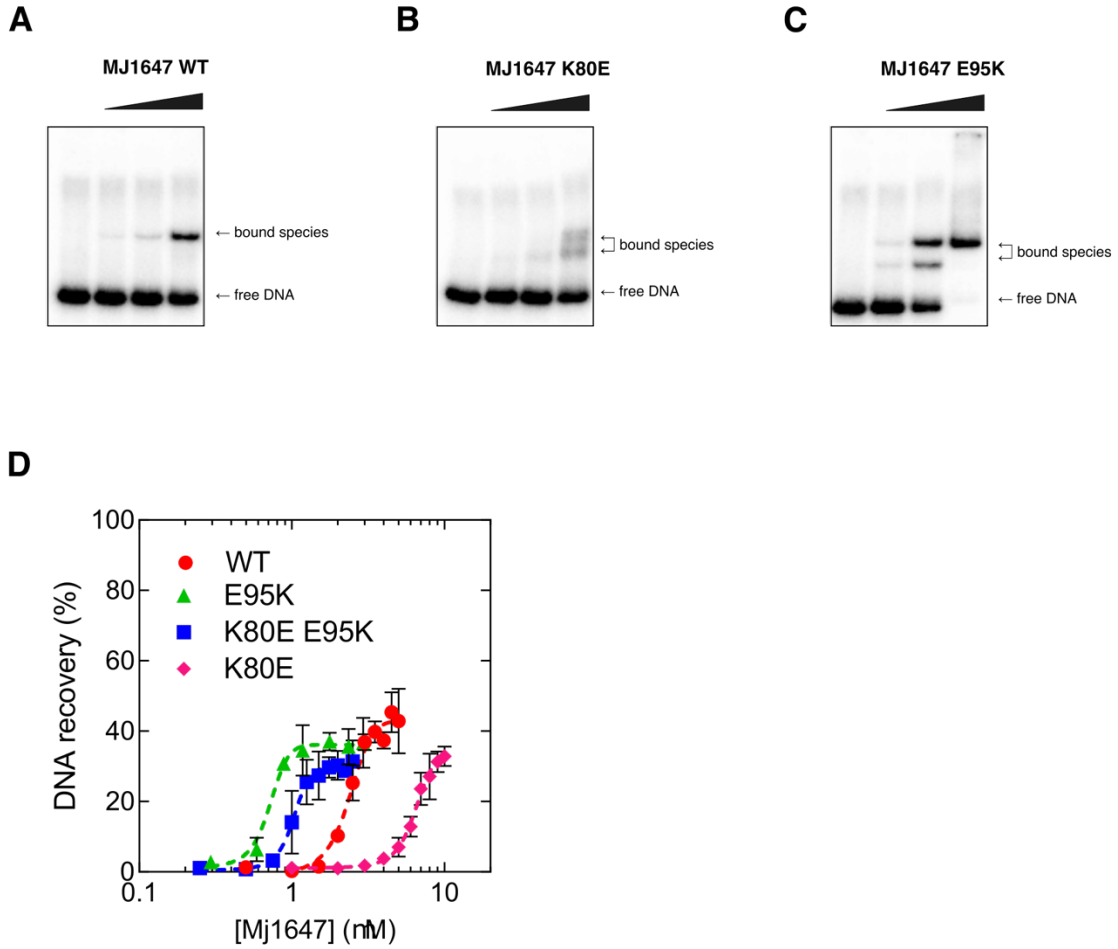
MJ1647 forms higher-order oligomers in the presence of DNA. Histone MJ1647 was subjected to cross-linking with BS3 in presence of increasing amounts of ~0.5kb DNA (0.25, 0.5 and 1 µg). Cross-linked samples were resolved by SDS-PAGE stained with protein-stain Sypro-Orange. The number of cross-linked monomers in each band are indicated in red with "n" denoting higher-order cross-linked complexes formed in the presence of DNA.



Supplementary Figure S3

(A) Predicted local Distance Difference Test (plDDT) for the five MJ1647 tetramer models produced by AlphaFold2

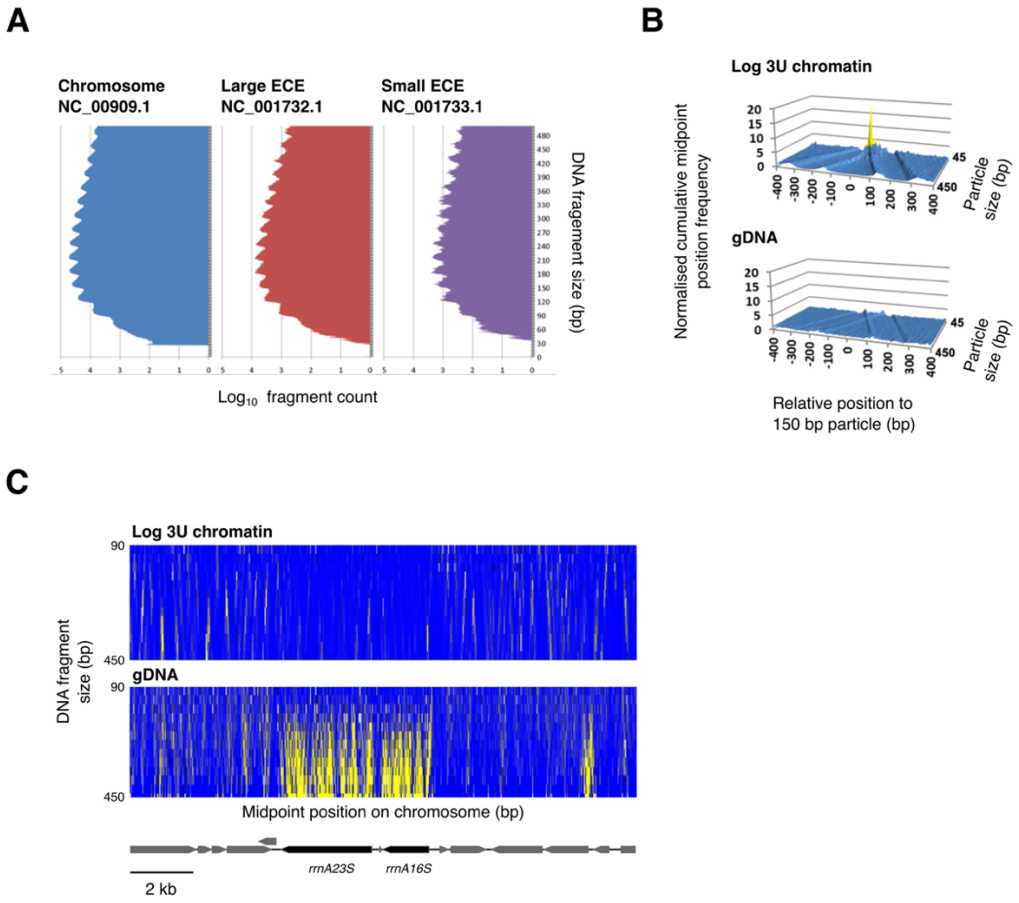
(B) Predicted alignment error (PAE) for the rank 1 MJ1647 tetramer model produced by AlphaFold2. A to D denote the four amino acid chains.



Supplementary Figure S4

(A-C) MJ1647 K80E and E95K bind to 60 bp DNA as dimer at lower protein concentrations. EMSA experiments testing binding of 0.04 to 0.16 µM MJ1647 WT (A), K80E (B), and E95K monomers (C) to 60bp 32P-labelled DNA.

(D) K80E and E95K mutants retain DNA bridging activity. DNA bridging assays with salt bridge mutants of MJ1647 K80E and E95K and the double mutant K80E/E95K compared to wild-type MJ1647. The protein concentration (monomer) is plotted against the recovery of radio-labelled prey DNA.



Supplementary Figure S5

(A) The M. jannaschii chromosome and extra-chromosomal elements (ECE) show similar bulk chromatinisation. Plots of number of reads versus paired-read insert size for Log3U data are shown separated according to genomic element.

(B) M. jannaschii MNase-resistant chromatin particles are not positioned in regular beads-on-a-string arrays. Genomic positions (n = 1618) of 150 bp particle midpoints (5 mers) scoring the top 1% of mid-point frequency within 5 bp chromosomal bins were mapped for the Log3U chromatin sample. Particle midpoint frequencies (y-axis) at and surrounding the 150 bp particle positions (x-axis; 5 bp bins) were then plotted across chromatin particle size (paired-end-read end-to-end distance) ranging from 45 bp to 450 bp (z-axis; 15 bp bins) for MNase-seq data from the log phase cells (upper panel) or de-proteinised genomic DNA (lower panel). Frequency values were normalised at each chromatin particle size to the average mid-point frequency per chromosomal bin within the x-axis window, to form a surface graph, as previously described [33] with values > 5 coloured yellow. No regular pattern of MNase-protection is visible within the chromatin graph (upper panel) beyond the presence of the original 150 bp-protected particles at the centre.

(C) rRNA loci are underchromatinised in M. jannaschii. MNase (3 units/ml) resistant mid-point frequency values from log-phase cells ranging in size from 90-450 bp in 30 bp intervals are plotted as a heatmap relative to a 16 kb region of the chromosome with positive frequency values coloured yellow. The rRNA operon genes are highlighted in black. The gDNA control shows increased signal across the rRNA operon likely due to the higher GC-content that counters the cutting preference of MNase.