# (A)Specific DNA binding of archaeal histones, the formation and positioning of hypernucleosomes

Erkelens, A.M.; Henneman, B.; Brouwer, T.B.; Kuijntjes, G.A.J.T.; Emmerik, C.L. van; Valk, R.A. van der; ... ; Noort, S.J.T. van

transcription factors loop DNA by *i)* binding to one location, *ii)* waiting for a spontaneous fluctuation in the DNA to cause a loop, and *iii)* stabilizing the loop by binding to a second location. However, unlike bacterial transcription factors that bind one at a time, protamine is known to coat the DNA, binding every 10 bp or so. Thus, the mechanism of protamine might be different from bacterial transcription factors. To study the mechanism of loop formation, we used a tethered particle motion (TPM) assay to measure the dynamic, real-time looping of single DNA molecules. We observed that folding did not occur in a single step as predicted. Instead, the DNA folded multiple times into long-lived (∼100 s), reversible, folded states. We used an atomic force microscopy (AFM) assay to image the folded structures directly. We observed that the partially folded molecules were partially folded loops—c-shapes or s-shapes—that had a radius of curvature of ∼10 nm. Analysis of the contours of the molecules suggest that protamine is bending the DNA rather than increasing the flexibility of the DNA. Thus, our model is that protamine loops DNA in multiple steps, bending it into a curved structure as it binds. This novel pathway for loop formation may be used by other multivalent cations to loop or bend DNA. For more information, see published work in *NAR* 2020.

**1531-Pos**

**(A)Specific DNA Binding of Archaeal Histones, the Formation and Positioning of Hypernucleosomes**

**Mandy Erkelens**[1], Bram Henneman[1], Thomas B. Brouwer[2], Gert-Jan A.J.T. Kuijntjes[2], Clara van Emmerik[3], Ramon A. van der Valk[1], Monika Timmer[1], Nancy C.S. Kirolos[1], Hugo van Ingen[3], John Van Noort[2], Remus T. Dame[1,4].
[1]Leiden Institute of Chemistry, Leiden University, Leiden, Netherlands, [2]Biological and Soft Matter Physics, Leiden University, Leiden, Netherlands, [3]Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, Netherlands, [4]Centre for Microbiol Cell Biology, Leiden University, Leiden, Netherlands.

Chromatin proteins are present in every species across the tree of life and ensure a dynamic structural and functional organization of their genome. The eukaryotic histone proteins H2A, H2B, H3 and H4 form nucleosomes and play a key role in gene regulation. Transcription is regulated via post-translational modifications of histone tails which induce changes in genome architecture. Many archaeal species express homologues of the eukaryotic histone proteins, but lack the functional tails. Also, their structure and function *in vivo* remains largely unknown. MNase digestion studies of archaeal chromatin suggested that histones form large structures on DNA consisting of an integer number of dimeric units. X-ray crystallography studies on the histone HMfB from *Methanothermus fervidus* bound to DNAsuggested the formation of an ''endless'' histone-core wrapped by DNA. This structure is called a ''hypernucleosome''. Here, we describe tethered particle motion (TPM) and magnetic tweezer (MT) experiments of both HMfA and HMfB from *M. fervidus* on a long DNA substrate in solution. We found cooperative binding of the histone proteins onto the DNA which resulted in strong DNA compaction. Based on the X-ray structure we mutated residues to disrupt proposed stacking interactions needed for hypernucleosome formation. This resulted in loss of cooperativity and a structure that is more easily disrupted by force, confirming the relevance of these interactions. Interestingly, these stacking mutant proteins were still able to recognize a specific DNA sequence (Clone20) generated via SELEX experiments. This result suggests that naturally occurring stacking-deficient histones could function as capstones by binding at natural high-affinity sites, marking the start and end of hypernucleosome structures. High-affinity sequences could thus play a role in positioning hypernucleosomes yielding a possible mechanism for histone-mediated transcription regulation.

**1532-Pos**

**Acidic Solutions to Archaeal Chromatin**

**Shawn Laursen**[1], Samuel Bowerman[2], Karolin Luger[2].
[1]Molecular, Cellular and Developmental Biology, Univ Colorado-Boulder, Boulder, CO, USA, [2]Dept Chemistry and Biochemistry, Univ Colorado Boulder, Boulder, CO, USA.

Cells must organize their genomes to provide compact structure and coordinate genome regulation. Like eukaryotes, most known archaea also encode histones, albeit with shorter sequences (∼68 amino acids) that span only the three-helix motif without additional domains. Most studies of histone structure-function relationships in archaea have focused on hyperthermophiles, such as *Thermococcus kodakorensis* and *Methanothermus fervidus*, where histones have been observed to wrap DNA into long super-helical ramps, referred to as archaeasomes. On the other hand, how histones function in other archaea is not well understood, especially in species that encode histones that have acidic isoelectric points. Normally, such acidic proteins would not be thought to bind nucleic acids. However, these histones maintain a basic ridge that may facilitate DNA binding despite their global acidic character. Here, we utilize biophysical assays and computational models to explore how acidic archaeal histones bind DNA and structure chromatin. Understanding how these archaeal species utilize acidic histones is essential for mapping how pressures from extreme environments has driven histone evolution and diversification.

**1533-Pos**

**Characterizing Partial Histone Wrapping States and the Histone-to-Protamine Transition in Sperm**

**Kyle M. Jones**, Yuxing Ma, Lynn Fu, Joanna M. Idrovo, Ashley R. Carter.
Physics, Amherst College, Amherst, MA, USA.

The proper packaging of DNA in sperm is absolutely necessary for human reproduction. Sperm nuclear DNA is tightly condensed, ensuring that the sperm is hydrodynamic and compact. This process is achieved using the sperm nuclear protein protamine, which directly or indirectly replaces the histones in somatic progenitor cells during spermiogenesis. Malfunctions of the histone replacement process are a known cause of male infertility. Our goal is twofold. We will examine partial histone wrapping states, which may play a key role in the histone replacement pathway. In particular, we will focus on comparing the prevalence of partially wrapped states between ''normal'' DNA and the 601 nucleosome positioning sequence that has special affinity for histones. Our second goal is to examine the mechanism of the histone-to-protamine replacement pathway. We hope to uncover both the physical mechanism behind histone replacement as well as the dynamics of the process. We will do so using a tethered particle motion (TPM) assay to measure the folding state in real time in addition to atomic force microscopy (AFM) that will allow us to image wrapping states. We will present our progress on these questions.

**1534-Pos**

**Regulation of *Provwx* Transcription By Local Chromatin Remodelling**

**Fatema Zahra M. Rashid**[1,2], Kathy R. Chaurasiya[1], Daan J.W. Brocken[1], Remus T. Dame[1,2].
[1]Leiden Institute of Chemistry, Leiden University, Leiden, Netherlands, [2]Centre for Microbial Cell Biology, Leiden, Netherlands.

Nucleoid Associated Proteins (NAPs) are architectural proteins that bind along the bacterial chromosome driving its compaction and organisation. They do so by lateral filament formation along the DNA, DNA bending, or DNA bridging, the latter of which results in the formation of long- and short-range DNA loops that determine the global and local structural organisation of the chromosome. NAP binding is sensitive to environmental changes such as fluctuations in temperature, pH, and osmolarity. Hence, NAPs organise the bacterial chromosome into a dynamic structure that is remodelled in response to changes in the cell environment. NAPs also function as transcription factors that coordinate global gene expression in response to environmental stimuli. This suggests that NAPs function as environmentally-sensitive transcription factors that regulate transcription by chromosome remodelling. *proVWX* is an osmosensitive operon of *Escherichia coli* that is repressed at low osmolarity and induced over 10 fold at high osmolarity. The expression of *proVWX* is regulated by H-NS — a NAP capable of lateral filament formation and bridging *in vitro*, with H-NS bridges functioning as transcription roadblocks. We have previously shown that H-NS is osmosensitive, such that, *in vitro*, high osmolarity conditions inhibit H-NS-mediated DNA bridging. We demonstrate structural remodelling of *proVWX* in response to high and low osmolarities *in vivo*. We use ensemble 3C-qPCR and single-molecule TIRF microscopy to demonstrate loop formation in the repressed *proVWX* operon and the loss of this bridging interaction in conditions that activate the operon.

**1535-Pos**

**Chromatin Remodeler CHD1 Forms Ternary Complexes with ADP and Pi Analogue which Mimic Transient States in ATPase Cycle**

**Md Noor A. Alam**[1], Kei Sadakane[2], Shinsaku Maruta[3].
[1]BioScience, Soka University, Hachioji, Japan, [2]Soka Univ, Hachioji, Japan, [3]Faculty of Science and Engineering, Soka University, Hachioji, Japan.

Chromatin remodeler Chd1 is one of the major types of ATP driven motor protein which moves along DNA, gaining energy from ATP hydrolysis and uses DNA as a 'Rail' to promote gene transcription by moving or ejecting the nucleosome. However, the motor mechanism by which this is done is still obscure at molecular level. In our study, we analyzed the properties of the Chd1-ADP-Pi analogues ternary complexes which mimic transient states in ATPase cycle for both the full length CHD1 (CHD1-FL) and catalytical motor domain of CHD1 (CHD1-MD) in order to clarify how ATP chemical energy transduced to motor activity. The catalytic domain of yeast CHD1 was expressed by using *E.coli* expression system and the full length CHD1 was expressed using *Bacteriophage* virus. Both of these full length and catalytic domain showed DNA stimulated ATPase activity which was significantly higher in case of CHD1-FL due to presence of the regulatory domain. In the presence of Pi analogues ($AlF_4^-$, BeFn, Vi), the Chd1-ADP-Pi analogues ternary complexes were formed and the DNA