Chapter 6

Summary and Conclusions

Understanding of chromatin organization and compaction in Archaea is currently limited. The genome of several megabasepairs long is folded by a set of small chromatin proteins to fit into the micron-sized cell. A first step in understanding archaeal chromatin organization is to study the action of individual chromatin proteins on DNA. Characterization of the architectural properties of these proteins is essential to understand how they shape and modulate the archaeal genome. This thesis describes the biophysical characterization of several chromatin proteins from the crenarchaeal model organism *Sulfolobus solfataricus*: Cren7, Sul7, Alba and Sso10a. The architectural properties of these proteins resemble those of their bacterial counterparts, suggesting that they could play a similar role in chromatin organization and global gene regulation.

A characterization of the architectural properties of Cren7 and Sul7 is described in **Chapter 2**. These small monomeric proteins are highly abundant in the cell and bind with no apparent sequence specificity to dsDNA. Cren7 and Sul7 are therefore believed to play an important role in the organization of the genomic DNA of *Sulfolobus* species. Although Cren7 and Sul7 are not conserved at the sequence level they are structural homologues and share biochemical and architectural properties. Both proteins induce a sharp bend upon binding to DNA. This bending angle is investigated using molecular dynamics simulations, which reveal that Cren7 and Sul7 both induce a non-flexible bend of ~ 50°. In depth analysis of DNA micromanipulation experiments confirms that the induced bends are non-flexible. In this respect these proteins differ from their bacterial and eukaryotic counterparts (HU and HMGB), which induce bends with a certain degree of flexibility. Single-molecule experiments reveal that Cren7 and Sul7 compact DNA molecules to a similar extent, by reducing the apparent persistence length. Interestingly, the protein-DNA interactions of Cren7 and Sul7 are unaffected by applied forces up to 3.5 pN as the proteins stay bound to the DNA at this force. This finding suggests that Cren7 and Sul7 are relatively stably bound to the DNA *in vivo*, which could be

important in the light of maintaining DNA integrity under the extreme environmental conditions of the habitat of thermophilic *Sulfolobus* species.

As *Sulfolobus* species live in extreme environments and are subjected to high temperatures and large temperature fluctuations it is important to know how DNA structure and protein-DNA interactions are affected by temperature. To this purpose we have developed a temperature-controlled tethered particle motion (TPM) set-up, which enables to investigate DNA structure and protein-DNA interactions at a single-molecule level at controlled temperatures (**see Chapter 3**). Using this technique we reveal that the intrinsic flexibility of dsDNA strongly depends on temperature in a range well below the DNA melting temperature. In the measured temperature range (23 – 52 °C) the apparent persistence length of dsDNA depends linearly on temperature and is slightly dependent on the composition of the DNA (i.e. the percentage of AT/GC base pairs). Besides the temperature effect on intrinsic DNA flexibility, our experiments reveal that temperature affects protein-DNA interactions of DNA-bending proteins in different manners. Temperature affects the binding affinity of Cren7 and Sul7 (static kink DNA-bending proteins), while it affects the distribution of bending angles induced by HU (a flexible hinge bending protein), rather than its binding affinity. The increase in flexibility of DNA at high – physiological relevant – temperatures and temperature dependent protein-DNA interactions of DNA-bending proteins suggest that high temperature contributes to a more compact configuration of the genomic DNA *in vivo*. In addition, as genome structure and topology are temperature dependent this may affect gene expression on a global level.

Chapter 4 describes a detailed characterization of the crenarchaeal chromatin protein Alba. *Sulfolobus* species express two different Alba proteins: Alba1 and Alba2. Alba2 forms obligate heterodimers with Alba1. The DNA-binding properties of these heterodimers differ significantly from Alba1 homodimers. Alba displays two different architectural modes: DNA-

bridging and DNA stiffening. Furthermore, binding of Alba1 is strongly cooperative due to dimer-dimer interactions, while Alba1:Alba2 heterodimers lack this cooperative binding behaviour. The lack of cooperative DNA binding of the Alba F60A mutant reveals that the F60 residue (which is not conserved in Alba2) plays an essential role in this cooperative binding behaviour. Interestingly, the bridging binding mode of Alba also depends on dimer-dimer interactions, including the F60 residue. Based on these findings we propose a model in which Alba dimers bound to DNA can alternate between two different orientations, facilitating either bridging of two DNA duplexes or side-by-side binding of dimers, resulting in stiffening. As Alba1:Alba2 heterodimers lack the F60 residue at one side of the dimer, they are unable to form long patches of closely packed dimers on the DNA. The ratio between Alba1 homodimers and Alba1:Alba2 heterodimers (controlled by expression levels of Alba1 and Alba2) can therefore tune the balance between stiffened and bridged DNA, which could provide mechanisms to regulate gene expression on a global scale *in vivo*.

The Sso10a protein is yet another DNA-binding protein that has been identified in *Sulfolobus* (**see Chapter 5**). Sso10a proteins exist as dimers with a coiled-coil dimerization domain with two DNA-binding domains at the ends. The DNA-binding domain of Sso10a adopts a helix-turn-helix (HTH) motif, which is often found in archaeal transcription regulators. *S. solfataricus* expresses three homologous Sso10a proteins: Sso10a1, Sso10a2 and Sso10a3, which are – based on transcription levels – expected to be abundant within the cell. Structural comparison of Sso10a1 and Sso10a2 reveals differences in the DNA-binding domains, which are expected to lead to different DNA-binding properties. An investigation on the architectural properties of Sso10a reveals three different binding modes: bending, bridging and stiffening. Both Sso10a1 and Sso10a2 induce bends into DNA by binding *in cis*, which leads to a decrease in the apparent persistence length. At relatively high protein concentrations Sso10a1 exhibits a second binding mode of binding, DNA-bridging, not observed for Sso10a2. Instead, Sso10a2 stiffens DNA at similar protein concentrations, which is likely due

to electrostatic dimer-dimer interactions. The multiple architectural properties of the Sso10a proteins suggest that these proteins function in shaping chromatin structure. The interplay between the different homologues and binding modes could provide mechanisms to dynamically shape the genome and to regulate gene expression *in vivo*.

With the characterization of the architectural properties of Cren7, Sul7, Alba and Sso10a, a first step has been made in understanding how crenarchaeal chromatin is organized. To further our understanding of the organization of the crenarchaeal nucleoid more information is needed on how these proteins act *in vivo* on different length scales within the complex cellular environment. A detailed understanding on the interplay of architectural proteins complemented with genome association studies could shed light on whether and how such interplay exist *in vivo*. Chromatin immunoprecipiation studies in combination with sequencing (CHip-seq) can provide information on whether different architectural proteins co-localize *in vivo* at specific sites along the genome, which could point to regulation mechanisms obtained by the interplay between these proteins. *In vitro* single-molecule studies could provide more detailed structural information on such nucleo-protein complexes formed by the combined action of different architectural proteins (eg. DNA-bending protein Cren7 or Sul7 with DNA-bridging protein Alba).

The numerous analogies between crenarchaeal and bacterial chromatin proteins suggest that higher-order organization of the crenarchaeal nucleoid relies on similar mechanisms as that of the bacterial nucleoid (**see Chapter 1**). Bridging proteins Alba and/or Sso10a could facilitate higher-order looped domains, similar to the bacterial bridging protein H-NS. Such looped domains could be further compacted by the action of DNA-bending proteins Cren7, Sul7 or Sso10a (see Figure 1.5). Information on higher-order structures *in vivo* could be obtained by applying chromosome conformation capture (3C) techniques, which allow to study the threedimensional genome structure at a high resolution. By probing

interactions between distant DNA segments this technique has been successfully applied in studies on *in vivo* genome structure in eukaryotes and bacteria (293). Combining 3C with chromatin immunoprecipitation (ChIP-loop) allows to study specific protein-mediated DNA-DNA interactions. Applying this technique could provide insights on how specific chromatin proteins are involved in higher-order organization of the crenarchaeal nucleoid and thus answer the question whether Alba and/or Sso10a are involved in higher-order organization by bridging distant DNA segments.

Integrating information from *in vitro* and *in vivo* studies at different scales will provide frameworks to obtain a detailed understanding on the structure and dynamics of the crenarchaeal nucleoid. Recent developments on genetic tools for archaea (294) enable *in vivo* studies, which have been unavailable for archaea until recently. Combining knowledge on structural and DNA-binding properties of chromatin proteins with information on genome-wide association and three-dimensional chromatin structure will provide a more detailed model on the organization and dynamics of the crenarchaeal nucleoid.