Ss-LrpB, a novel transcriptional regulator from *Sulfolobus solfataricus* P2

Although Archaea are true prokaryotes, transcription in these organisms resembles the eukaryotic process very closely. On the other hand, many transcription regulators appear to be Bacteria-like. How do these regulators function? This question remains largely unanswered, since information on archaeal transcription regulation is scarce. Many characterized archaeal species are extremophiles, including the hyperthermoacidophile *Sulfolobus solfataricus* P2, which grows optimally at 80°C and pH 2-3. In this organism, we identified a transcription regulator belonging to the bacterial/archaeal Leucine-responsive Regulatory Protein family (Lrp), called Ss-LrpB. The aim of this thesis was to characterize this protein, unravel its function (DNA-binding mode, physiological role) and structure as a contribution to a better understanding of archaeal transcriptional regulation.

Ss-LrpB binds its own control region *in vitro* at three regularly spaced, 15 bp-long binding sites, which is an observation suggestive of autoregulation. Each binding site is bound by one Ss-LrpB dimer. The two outer binding sites are bound with a higher affinity than the weaker, middle binding site which is only bound at higher Ss-LrpB concentrations. This interaction is highly cooperative. Atomic Force Microscopy allowed us to visualize Ss-LrpB-DNA complexes with a different stoichiometry and confirmed exhaustive DNA deformations upon protein binding, and even DNA wrapping when all three binding sites are occupied. The three binding sites appear to have a well conserved sequence. The deduced palindromic consensus sequence was the starting point of an extensive analysis (saturation mutagenesis) of *in vitro* binding to mutated consensus binding sites. This resulted in a detailed knowledge of the DNA-binding specificity of Ss-LrpB and the elaboration of an energy normalized sequence logo. The latter is a valuable tool for the search of new binding sites and thus Ss-LrpB regulon members in the genome of *S. solfataricus* P2. Several promising potential targets of the regulator have already been identified, including a *porDAB* operon, encoding a pyruvate ferredoxine oxidoreductase, a potential transporter gene, a methylthioadenosine phosphorylase gene and a conserved gene of unknown function. Finally, we also obtained crystals of the C-terminal domain of Ss-LrpB, which is predicted to be a αβ-sandwich (βαββαβ-fold). This domain, also called Regulation of Amino acid Metabolism (RAM) domain, is responsible for oligomerization and ligand binding in other Lrp-like regulators. A data set was collected at 2 Å resolution.