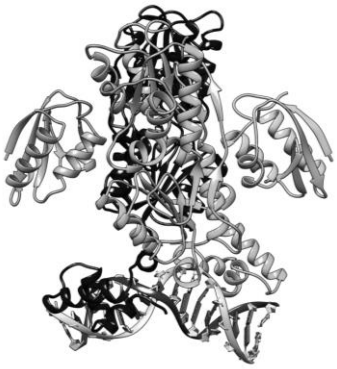
Investigation of the Protein-DNA Binding Mechanism of Carbon Catabolite Protein A

Achim Sandmann, Christophe Jardin, Heinrich Sticht

Bioinformatik, Institut für Biochemie, Friedrich Alexander University, Fahrstr. 17,

91054 Erlangen, Germany

The global regulator Carbon catabolite Protein A (CcpA) controls carbon metabolism in *Bacillus subtilis*. It does so by binding to the degenerate consensus site WTGNNARCGNWWWCAW [1]. To investigate how CcpA can bind to such diverse sequence motifs, we tried to identify contributions to binding selectivity. On the one hand, direct contacts via hydrogen bonds and nonpolar interactions to the nucleotide bases ('base readout') strongly influence the preference of proteins for specific sequences. On the other hand, the base composition also influences the shape and flexibility of the DNA, thereby modulating the strength of the interactions.



The strongest conserved bases of the consensus site are the central CG bases, at which the DNA is bent in the complex structure. The direct contacts of the bases are hydrogen bonds with the CcpA backbone via a guanine amine group in the centre of the minor groove and two intercalated leucine side chains. In addition, CG represents a pyrimidine-purine base step, which is known to facilitate kinks, which might favour shape readout at this site.

To dissect the individual contributions of base and shape readout, the CcpA-DNA complex was simulated as wild type and compared to a mutant, in which the central CG base step was replaced by GC. Interestingly, the CcpA-DNA hydrogen bonds remained stable throughout the simulation of the mutant, whereas MMPBSA analyses showed a higher energy requirement to bend the mutant DNA sequence.

[1] M. A. Schumacher, M. Sprehe, M. Bartholomae, W. Hillen, R. G. Brennan, *Nucleic acids research* **2011**, *39*, 2931-2942.