that is synthesized in the cytoplasm of living organisms of the three domains: archaea, bacteria, and eukarya. Glycogen synthase (GS), a central enzyme in glucose metabolism, catalyzes the successive addition of α-1,4-linked glucose residues using UDP-Glc or ADP-Glc as the glucosyl donor to the non-reducing end of a growing glycogen molecule. Many of the enzymes that are involved in the glycogen metabolism possess in addition to their catalytic sites, distinct non-catalytic carbohydrate binding modules (CBMs), or specific glycogen-binding sites, which provide these enzymes with high affinity for the polysaccharide.

In this work we identify and structurally characterize a glycogen-binding site present on the surface of the GS from the archaeon *Pyrococcus abyssi* (*Pa*GS) and show that this site binds glycogen with high affinity. *Pa*GS is a homotrimeric protein in which each subunit exhibits the characteristic GT-B fold: two Rossmann-fold domains with the catalytic center located in a deep cleft between them. Crystallographic analysis of the complex between maltohexaose and a monomeric form of *Pa*GS shows that the oligosaccharide binds at the N-terminal domain of the enzyme, curling around the lateral chain of Tyr174. The location of the glycogen-binding site within the overall structure of *Pa*GS is similar to one of the malto-oligosaccharide-binding sites recently described for the *Escherichia coli* GS (*Ec*GS) [1] and, most interestingly, to the glycogen-storage site in glycogen phosphorylase (GP). We show that this glycogen-binding site is functionally conserved in eukaryotic GSs. The disruption of this binding site in both the archaeal and the human muscle glycogen synthases has a large impact when glycogen is the acceptor substrate. Instead, the catalytic efficiency remains essentially unchanged when small oligosaccharides are used as substrates. Mutants of the human muscle enzyme with reduced affinity for glycogen show also an altered intracellular distribution and a marked decrease in their capacity to drive glycogen accumulation *in vivo*.

The presence of a high-affinity glycogen-binding site away from the active centre explains not only the long recognized strong binding of GS to glycogen but also the processivity and the intracellular localization of the enzyme. These observations demonstrate that the glycogen-binding site is a critical regulatory element responsible for the *in vivo* catalytic efficiency of GS [2].

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Structure study of UHRF1.

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Post-translational modifications of histone proteins and cytosine methylation of genomic DNA are major epigenetic traits and regulate various chromatin-template processes in mammals. The pattern of these epigenetic traits is cooperatively established and inherited during the cell cycle. UHRF1 (also known as Np95 or ICBP90) is thought to play an important role in linking the two major epigenetic traits. It recognizes hemi-methylated DNA generated during DNA replication through interactions with its SRA domain, [1], [2]. UHRF1 also contains linked histone reader modules, a tandem tudor domain and a PHD finger and interacts with histone H3 containing tri-methylated Lys 9, [3].

To obtain the underlying mechanism of the epigenetic marks recognition by UHRF1, we determined the crystal structures of the unliganded SRA domain and its complex form with hemi-methylated DNA at 1.8 Å and 1.6 Å resolution, respectively. The structure showed that the DNA binding caused a loop and an N-terminal tail of the SRA domain. The methyl-cytosine base at the hemi-methylation site was flipped out from the DNA helix, which has not seen in other DNA binding proteins. We also determined the crystal structure of linked histone reader modules region of UHRF1 in complex with the amino-terminal tail of histone H3 at 2.9 Å resolution. The structure indicated that the inter-module linker plays an essential role in the formation of histone H3 binding hole between the reader modules. Our data revealed how multiple histone modifications were simultaneously decoded by the linked histone reader modules of UHRF1.

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Structure-based drug design of selective 5'-nucleotidases inhibitors

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The monophosphate 5'-nucleotidases, including 5'(3')-deoxyribonucleotidase, belong to a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates. The ribonucleotides and deoxyribonucleotides can be synthesized *de novo* from low-molecular-weight precursors or by salvage from nucleosides or nucleobases produced in catabolism of nucleic acids[1]. In this salvage pathway, ribonucleotides and deoxyribonucleotides are phosphorylated by nucleoside and nucleotide kinases to maintain sufficient pools of dNTP's and NTP's for synthesis of DNA and RNA, respectively. The phosphorylation by cellular nucleoside kinases is opposed by 5'-nucleotidases that dephosphorylate ribo- and deoxyribonucleoside monophosphates[2], [3], [4]. Besides their role in the regulation of physiological dNTP pools, substrate cycles between ribonucleotidases and kinases may affect the therapeutic action of pyrimidine nucleoside analogs used as anticancer and antiviral agents. Such compounds require the nucleoside kinases activity for phosphorylation to their active forms. Results of clinical and *in vitro* studies propose that an increase in nucleotidase activity can interfere with nucleoside analogue activation resulting in drug resistance [5].

The main goal of this project is the search for potent and selective inhibitors of mammalian 5'-nucleotidases based on nucleoside phosphonic acids and their derivatives and comparison of sensitivity of 5'-nucleotidases isolated from various sources toward individual inhibitors.

We have prepared 2 types of human 5'-nucleotidase: cytosolic and mitochondrial by recombinant expression in E. coli. Two strategies of finding potential inhibitors are used. First, a random series of nucleoside phosphonic acids derivatives are tested. Second, testing of rationally designed compounds based on a published structure of