obtained by irradiating the crystals for 10 min. with a blue laser (508 nm). Diffraction data were collected at the PXII beamline of the Swiss Light Source at 100K using a MarResearch CCDC detector.

The structures were solved in space group $P2_12_12_1$ by molecular replacement using the monomer of Dronpa (pdb code 2z1o) as search model. The asymmetric unit contains 12 monomers, associated in three tetramers. The structures were refined at an effective resolution of 3.0 Å for the *on*-state and 3.15 Å for the *off*-state. R/R_{free} values converged at 21.1/25.7% for the *on*-state and 21.3/25.0% for the *off*-state.

PDM1-4 exhibits a β-barrel structure typical for GFP-like proteins. Furthermore, a light-driven *cis-trans* isomerization of the chromophore is observed. From the structures of the *on*- and *off*-states we elucidate that the presence of nickel ions, interacting with His 194 and His 212, decreases the flexibility of the β-strands, resulting in the slower switching kinetics of PDM1-4. The photoswitching mechanism of reversible photoswitchable fluorescent proteins not only arises from the flexibility of the chromophore accompanied by a rearrangement of the proximate residues, but is also influenced by the flexibility of the β-strands.

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Keywords: fluorescence, nickel, protein

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Crystal Structures of Bacterial Peptidoglycan Amidase AmpD and an Unprecedented Activation Mechanism

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AmpD is a cytoplasmic peptidoglycan (PG) amidase involved in bacterial cell-wall recycling and in induction of *β*-lactamase, a key enzyme of *β*-lactam antibiotic resistance [1], [2]. AmpD belongs to the amidase_2 family that includes zinc-dependent amidases and the peptidoglycan-recognition proteins (PGRPs), highly conserved pattern-recognition molecules of the immune system [3], [4]. Crystal structures of *Citrobacter freundii* AmpD were solved for the apoenzyme, for the holoenzyme at two different pH values, and for the complex with the reaction products, providing insights into the PG recognition and the catalytic process. These structures are significantly different compared to the previously reported NMR structure for the same protein [5]. The NMR structure does not possess an accessible active site and shows the protein in what is proposed herein as an inactive "closed" conformation. The transition of the protein from this inactive conformation to the active "open" conformation, as seen in the X-ray structures, was studied by targeted molecular dynamics simulations, which revealed large conformational rearrangements (as much as 17 Å) in four specific regions representing one third of the entire protein. It is proposed that the large conformational change that would take the inactive NMR structure to the active X-ray structure represents an unprecedented mechanism for activation of AmpD. Analysis is presented to argue that this activation mechanism might be representative of a regulatory process for other intracellular members of the bacterial amidase_2 family of enzymes.

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Keywords: crystal structure, cell-wall recycling, conformational change

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Structure of a novel bacterial small molecule sensor domain with two ligands

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Chemoreceptors play important roles in the adaptation of bacteria to changing environmental conditions. For most chemoreceptors the cognate signal molecules are unknown. Receptors can be classified according to the size of their ligand-binding domain (LBD) into cluster I and cluster II. Several structures of cluster I domains are available, but structural information on the larger cluster II domains is lacking. Here we report the structure of *P. Putida* McpS-LBD in complex with two strong chemoattractants, succinate and malate. Crystals were initially grown using the counter-diffusion method to be further improved by the vapor diffusion technique. Malate crystals diffracted up to 1.8 Å and the structure was solved by wavelength Anomalous Scattering (SAS) using Se-methionine derivatized protein. The McpS-LBD structure together with succinate was solved at 1.9 Å resolution by Molecular Replacement (MR). In the solved structures McpS-LBD is a dimer in which each monomer folds as 6 α-helices (2 long and 4 short) organized in two four-helix bundles located at opposite sites or their long bundle axis.These structures are the first examples of cluster II domains and identifies McpS-LBD as a novel small molecule binding domain. Moreover, despite low sequence similarity, both bundles share high structural similarities with *E. Coli* Tar-LBD. In McpS-LBD, as in the case of Tar, the binding of ligand molecules is accomplished by amino acids from both monomers. Site-directed mutagenesis of amino acids involved in ligand binding causes loss of binding activity. It is proposed that Tar-LBD and McpS-LBD are the result of convergent evolution.

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Keywords: ligand binding domain, chemoreceptor, McpS

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Non-catalytic glycogen-binding site and its functional role in glycogen synthase

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Glycogen is a polymer of α-1,4- and α-1,6-linked glucose residues

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