spacer- and RPEL-actins, which bind weakly in solution. Cytoplasmic MRTF-A localization in resting fibroblasts requires spacer-actin binding. The bipartite importin {alpha-beta} binding site is buried in the pentameric assembly, explaining how elevated G-actin concentrations prevent MRTF-A nuclear import. The structures show how actin loads onto the RPEL domain, and reveal a molecular mechanism by which actin can control the activity of one of its ligands.

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Keywords: actin, structure, MRTF-A

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The Molybdenum Storage Protein – a special kind of metallo-protein

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The diazotrophic soil bacterium *Azotobacter vinelandii* utilizes a FeMo-cofactor containing nitrogenase to accomplish nitrogen fixation. As nitrogenase is required in large amounts, the cell extracts a lot of molybdenum (Mo) from the environment and stores it in a special Molybdenum Storage Protein (MoSto)[1] as polyoxometalate clusters[2]. MoSto crystallizes in a needle-like shape. Best crystals diffract to 1.6Å resolution. An X-ray crystal analysis of the Mo-loaded MoSto revealed different types of Mo-oxide based clusters some being covalently bound while others are not. These clusters are synthesized in an ATP-dependent process whose mechanism is not yet known. *In-vitro* experiments showed that it is possible to entirely deplete MoSto from its metal clusters and subsequently reload it again. The depletion is a pH-driven triphasic process which can be varied with temperature and time of incubation [1]. MoSto can load up to 120 atoms per protein molecule. Further research is necessary to determine the mechanism how the clusters are built from monomolybdates and how their selective release from MoSto is organized.

[1] J. Schemberg *et al*., *ChemBioChem* **2008**, *9,* 595-602. [2] D. Fenske *et al*., *ChemBioChem* **2005**, *6,* 405-413

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Features critical for membrane binding revealed by DivIVA crystal structure

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DivIVA is a highly conserved protein in Gram-positive bacteria that localizes at the poles and division sites, presumably through direct sensing of membrane curvature. The correct localization of the protein is essential for its biological function, which involve both the correct placement of the septum at midcell and also the segregation of the DNA into the pre-spore during sporulation. DivIVA deletion causes filamentous growth in *Bacillus subtilis*, whereas overexpression causes hyphal branching in *Streptomyces coelicolor*. The mechanism of subcellular DivIVA targeting and function remain unknown. We present data that demonstrates that DivIVA binds directly to bacteria membrane and explains how this protein shows a multifunctional behavior.

We prove that DivIVA is a two independent domains protein linked by a flexible tag and both domains together are essential to fulfill protein localization and function. The crystal structure determination shows that both domains share a parallel coiled-coil folding. Surprisingly the N-terminal domain also includes crossed and intertwined loops between the two coiled-coil molecules, which is completely new and is involved in protein targeting in combination with the presence of a key hydrophobic residue surrounded by positively charged residues. To clarify N-terminal domain function in DivIVA membrane targeting we have used an *in vivo* and *in vitro* approach, which shows Phe17 and its chemical context in DivIVA structure, is essential for membrane binding. We propose that the hydrophobic residues insert into the membrane and that the positively charged residues bind to the membrane surface. Also low-resolution crystal structure of the C-terminal domain displays a curved tetramer made from two parallel coiled-coils, which is the lowest oligomerization state of DivIVA showed by AUC. The N-terminal and C-terminal parts have been merged into a model of the full length, 30nm long DivIVA protein. Our experiments suggest that full-length DivIVA is an elongated tetramer with membrane targeting residues at both ends, which increase the protein ability to bind to membranes and other proteins.

Keywords: bacterial cell division, peripheral membrane protein, membrane curvature

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Structural basis for complement Factor I control and its disease associated sequence polymorphisms

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The complement system is a key component of innate immune defence, coordinating inflammation with innate and adaptive responses, both in infection and in homeostasis. Complement regulation is critical for prevention and control of disease.

We have determined the crystal structure of the complement regulatory enzyme human Factor I (fI). Factor I is seen to be in a proteolytically inactive form, demonstrating that it circulates in a zymogen-like state, despite being fully processed to the mature sequence.

Mapping of functional data from mutants of fI onto the structure suggests that this inactive form is maintained by the non-catalytic heavy chain allosterically modulating activity of the light chain.

Once the ternary complex of fI, a cofactor and a substrate is formed,

the allosteric inhibition is released and fI oriented for cleavage.

In addition to explaining how circulating fI is limited to cleaving only C3b/C4b, our model explains the molecular basis of disease-associated polymorphisms in fI and its cofactors.

Keywords: complement, factor I, allostery

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Protein complex structures inform about the reactivity of the hemoprotein MauG

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Many proteins require organic transformations of select amino acid residues for maturation. The enzyme methylamine dehydrogenase (MADH) is an example whereby oxygen-atom insertion and covalent linking of specific residues produce its cofactor, TTQ (tryptophan tryptophyl quinone). The hemoprotein MauG catalyzes TTQ biosynthesis. While the sequence of modifications in TTQ synthesis is unknown, the final step is hypothesized to be the oxidation of quinol-TTQ to the active quinone form. X-ray crystal structures of wild type and point-mutant forms of MauG in complex with various forms of its large protein substrate MADH have provided insight into the reactivity of MauG.

Crystallization of MauG has been achieved in complex with a precursor-form of MADH, preMADH, its native substrate. The crystal structure revealed large separations between the two *c*-hemes of MauG and nascent TTQ site of preMADH. Biochemical studies and spectroscopy indicated that the reactive form of MauG during turnover is an unusual bis-Fe(IV) intermediate. Unlike established paradigms for direct oxygen-atom transfer from an Fe(IV)=O heme, the MauG/preMADH complex uses long range electron-transfer in TTQ formation. TTQ formation occurred *in crystallo* upon addition of hydrogen peroxide, indicating that MauG could catalyze cofactor synthesis within the crystallized complex.[1] The processive nature of TTQ synthesis in the MauG/preMADH complex has been further examined. The quinol-MADH and quinone-MADH forms also co-crystallize with MauG and represent a reaction intermediate and the product form, respectively, for TTQ synthesis. The structures of the complexes with the three forms of MADH are essentially identical, and collectively suggest that the MauG/MADH complexes represent the physiological form that remains intact during turnover.

The X-ray crystal structure of MauG/preMADH enabled identification of potential electron transfer chain components. Point mutants at a MauG Trp residue implicated in the reaction pathway rendered the enzyme inactive. However, these mutants retained the native fold of MauG and co-crystallized with preMADH. The high degree of similarity between their X-ray crystal structures and that of MauG/preMADH confirmed that disruption of the electron transfer process results in loss of TTQ synthesis activity. Point mutants at the unusual His/Tyr-ligated heme of MauG also resulted in loss of native reactivity, suggesting a critical role for Tyr at the six-coordinate heme in stabilizing the Fe(IV) state of the reactive MauG intermediate.[2]

The bis-Fe(IV) MauG intermediate has been characterized in solution by XAS. Spectral comparisons with the Fe(IV)=O form of cytochrome c peroxidase have confirmed the presence of a short Fe-O bond in high valent MauG. The full spectral analyses for MauG solution samples will enable application of this technique to single crystal samples in future characterization of the bis-Fe(IV) MauG intermediate.

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Keywords: X-ray_diffraction, hemoprotein, mutants

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Structures of glucoside hydrolases from human gut microbiome

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Metagenomics aims to analyze, reconstruct and characterize how microbial communities organize and evolve their collective genomes to thrive in the environments they co-habit. The human intestine harbors a large number of microbes forming a complex microbial community that greatly affects the physiology and pathology of the host. Many of which are uncharacterized or unculturable. The human gut microbiome represents a highly complex microbial community that has a significant impact on human health. The gut microbiome performs a diverse set of functions supplementing those performed by the host and is believed to significantly enhance the metabolism of amino acids, carbohydrates, xenobiotics, methanogenesis, biosynthesis of vitamins and other compounds. Therefore, microbiomes represent a potential pool of genes coding for novel proteins and functions. In the human gut microbiome, the enrichment in certain protein gene families appears to be widespread. They include enzymes involved in carbohydrate metabolism such as glucoside hydrolases of dietary polysaccharides and glycoconjugates. The Midwest Center for Structural Genomics (MCSG) (a component of Protein Structure Initiative:Biology project) targets proteins from microbes found in human gut microbiome in healthy and diseased individuals. The MCSG structure determination pipeline is being applied to the microbial proteins that emanate from these projects. We have analyzed gut metagenomic data to identify novel, previously uncharacterized, human microbiome-specific protein families. The analysis revealed an abundance of carbohydrate metabolizing enzymes. A set of reagent genomes was used to extract genes from gut microbiome and produce proteins for structural studies. A number of structures from normal and enteric microflora have been determined. The structural and functional results will be discussed.

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Keywords: Glucoside Hydrolases, Human Microbiome, Enzyme Specificity

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Structure of FoxE, a Fe(II) oxidoreductase involved in anoxygenic photosysnthesis

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