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.

[1] L.M.R. Jensen, R. Sanishvili, V.L. Davidson, C.M. Wilmot, *Science* **2010,** *327,* 1392. [2] N. Abu Tarboush, L.M.R. Jensen, M. Feng, H. Tachikawa, C.M. Wilmot, V.L. Davidson, *Biochemistry* **2010**, *49,* 9783.

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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FoxE is a protein belonging to the foxEYZ operon of sp. Strain SW2. This protein is known to confer light-dependent Fe(II) oxidation activity by itself and thus to allow for Fe(II) based anoxygenic photosynthesis[1]. This protein is estimated have 25kDa (259 residues) and two haem-c binding sites. It is believed to reside on theperiplasm and to stimulate Fe(II) oxidation directly by serving as an Fe(II)oxidoreductase.

Crystallization trials of FoxE were performed on a robot using nanoscale drops andcommercial crystallization screens. Best diffracting crystals were obtained at 293 K in 1.2 M sodium/potassium phosphate pH=7 with copper chloride as additive. Crystals were cryo protected by plunging them into mother liquor containing 25% v/v glycerol. Diffraction data was recorded at ESRF at 100 K in beamline ID23-1/2, using radiation near the iron absorption edge.

The collected dataset was processed with XDS, the phase problem was solved by Single Anomalous Dispersion method (SAD) using the HKL2MAP and the SHELXC/D/E suite.

FoxE crystals belonged to the spacegroups P3121 (trigonal) and P43212 (tetragonal). The trigonal crystal diffracted to an higher resolution with up to 2.4Ã… resolution (I/sigI=1.9 at the highest resolution shell, 2.54-2.44Ã…). The data set obtained had a global multiplicity of 32.2, Rsym=13% and Rpim=3%. The collected data contained anomalous signal up to 3.7Ã… resolution (self-anomalous CC above 30%), and since six centres were found in the anomalous substructure, one could deduce that the crystal asymmetric unit contained three FoxE molecules (crystal solvent content of 52%).

SHELXE was used to trace the main chain of the protein (610 out of 777 in the asymmetric unit). Coot and phenix were used for real and reciprocal space refinement (NCS and TLS restrains used) with a final Rwork/Rfree of 0.2227/0.2397. The final model of the trimer presents good stecheometry and only four Ramachandran outliers, as assessed by MolProbity. The Trimer formation implies 31% of surface area occlusion and the minimum distance between redox centres (two hemes and one disulfur bridge) is ca. 18 Angstroms.

[1] L.R. Croal, Y. Jiao, D.K. Newman. *Journal of Bacteriology* **2007**, *189,* 1774-1782

Keywords: photosynthesis, heme, SAD

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Crystal structure of zinc-finger domain of Nanos and its functional implications

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Translational control of mRNAs is crucial in developmental processes including cell division, cell-fate determination and embryonic axis establishment in early embryogenesis. Most types of translational control are mediated by a sequence in the 3' untranslated region (3'-UTR) and are achieved by the interaction of various regulatory factors such as RNA-binding proteins. Nanos is a highly conserved RNA-binding protein in higher eukaryotes and functions as a key regulatory protein in translational control using a 3'-UTR during the development and maintenance of germ cells. In combination with Pumilio, Nanos represses the translation of maternal hunchback mRNA in the early *Drosophila* embryo, thereby governing abdominal segmentation. Nanos and Pumilio also have a variety of functions in the primary germ cells (PGCs). Nanos is essential for the development of PGCs. One of the regulatory targets of Nanos and Pumilio in PGCs is thought to be Cyclin B mRNA, Pumilio and Nanos directly bind to an element in the 3'-UTR to repress its translation. Although studies have revealed important functions of Nanos, neither the atomic structure of Nanos nor the structural basis of the interaction between Nanos and RNA has been reported.

In this study, we present the first, to our knowledge, crystal structure of the zinc-finger domain of zebrafish Nanos (residues 59–159), which includes the two conserved zinc-finger motifs. Our study also reveals that the two CCHC motifs actually bind zinc ions and that the zinc-finger domain of Nanos adopts a novel structure. Furthermore, we reveal a conserved basic surface that is responsible for RNA binding. Our results provide the structural basis for further studies to clarify Nanos function.

[1] H. Hashimoto, K. Hara, A. Hishiki, S. Kawaguchi, N. Shichijo, K. Nakamura, S. Unzai, Y. Tamaru, T. Shimizu, M. Sato, *EMBO Rep.*, **2010**, *11*, 848-853.

Keywords: structure, protein, RNA

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Structural Characterization of the [Co^{II}(TPP)(18-C-6)$_2$] Complex

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This work concerns the synthesis, the spectroscopic and the X-ray molecular structural characterization of a new six-coordinated cobalt(II) porphyrin derivative: bis(18-C-6) (tetraphenylporphyrinato)cobalt(II) complex ([Co^{II}(TPP)(18-C-6)$_2$]). The UV-visible spectrum of this species presents a Soret band at 412 nm and the IR spectrum exhibits a strong stretching frequencies at 1102 cm^{-1} attributed to the ν(O-C) of the ether crown 18-C-6.

The X-ray structural analysis shows that the complex [Co^{II}(TPP)(18-C-6)$_2$] crystallizes in the triclinic system (space group P-1). Crystal data for this complex: a = 10.262(2) Å, b = 11.250(2) Å, c = 11.815(2) Å, α = 104.180 (0) °, β = 106.100 (0) °and γ = 108.489 (0) °, Z= 2, R_1=0.0056, WR_2=0.181 and S=1.042. The title compound is a polymer where the cobalt(II) is bonded to two oxygen atoms of two trans coordinated ether crown 18-C-6. The Co^{II}—O(18-C-6) is 2.406 (2) Å is slightly longuer than the related species bis-THF derivative [Co^{II}(F_{28}TPP)(THF)$_2$] (octafluoro-5,10,15,20-tetrakis(pentafluorophenyl)porphinato) [1] for which the distance Co^{II}—O(THF) = 2.31(1) Å.

[1] R.Crescenzi, E.Solari, C. Floriani, A.Chiesi-Villa, C.Rizzoli *Inorg.Chem.* **1996**, *35,* 2413.

Keywords: cobalt(II) porphyrin, Bis(18-crown-6) cobalt complexe

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Refinement of the Vault particle using DEN protocols with NCS constraints

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