Session V - Saturday, September 26

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CHARACTERIZATION AND MODULATION OF MITOCHONDRIAL SIRTUINS 4 AND 5

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Sirtuins are NAD^+-dependent protein lysine deacylases involved in the regulation of, e.g., genomic maintenance or metabolism [1]. Furthermore, they are linked to lifespan-extension and the delay of aging-associated diseases [2]. Mammalian cells bear seven different isoforms, which are either located in the cytoplasm (Sirt2), nucleus (Sirt1/6/7), or mitochondria (Sirt3/4/5) [1]. Their conserved catalytic core is composed of a Rossmann-fold and a Zinc-binding module, with the active-site cleft in between [3]. Whereas Sirtuins show only moderate specificity for the sequence of their polypetide substrates, the type of acyl-modification on the substrate lysine is changing kinetics dramatically. Sirt1, 2 and 3 are robust deacetylases, Sirt5 was shown to have strong desuccinylase activity [4], and Sirt6 is effective against long-chain fatty acyls [5]. In contrast, Sirt5 and 6 are barely measurable deacetylases, all of which is due to different binding motifs in the acyl-lysine binding site.

To improve our understanding of physiological acyl substrates of Sirt5 and to exploit its acyl selectivity for the development of first Sirt5 specific inhibitors, we analyzed the molecular details of the interaction between Sirt5 and its substrate acyl. For this purpose, we screened a library of differently acylated peptides as substrates or inhibitors and characterized them kinetically and structurally. We find that glutarylated substrates are deacylated even more efficiently than succinylated substrates, possibly due to a tensed helical geometry ion in the substrate acyl when accommodated in the Sirt5 active site [4]. We further found inhibitory acyl modifications allowing us, e.g., to exploit simultaneously Sirt5 specific features and the conserved, affinity-providing nicotinamide binding site, resulting in first Sirt5 specific inhibitors that can serve as leads for drug development [4]. Since no information on acyl specificity and active site features are available yet, we started to characterize it structurally and biochemically using similar approaches. Using our panel of acyl peptides, we found acylations that are readily removed by Sirt4 and used a Sirt4 homology model for rationalizing these findings. We now employ these acyl peptides as stabilizing ligands for our efforts to crystallize Sirt4 and for our searches for first Sirt4 specific inhibitors.

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STRUCTURAL ANALYSIS OF YrdC, RESPONSIBLE FOR ACTIVATION OF THE N^6-THREONYLCARBAMOYL MOIETY IN t^6A37 tRNA MODIFICATION

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Over 100 distinct tRNA modifications have been characterized. Only a few modifications are universally conserved, one of them leading to N^6-threonylcarbamoyl adenosine (t^6A) found at position 37 of ANN decoding tRNAs [1]. YrdC/ Sua5 and Kae1, a central component of the KEOPS/ EKC complex, have recently been shown to be involved in t^6A biosynthesis [2]. The base modification t^6A37 is crucial to translational accuracy, explaining mutants in YrdC/ Sua5 and Kae1 lead to pleiotropic effects in cells including defects in transcription and genome stability [3]. While previous studies have demonstrated that for t^6A37 biosynthesis threonine, bicarbonate, ATP and tRNA are required; the exact roles of YrdC/ Sua5 and Kae1 in this process have remained unclear.

YrdC-like and Kae1-like domains are found in the tobramycin 6"-*O*-carbamoyltransferase TobZ from *Streptoalloteichus tenebrarius* as well as the [NiFe]-hydrogenase maturation factor HypF from *E.coli*. The structure of TobZ and its ATP-dependent antibiotic modifying reaction mechanism was previously elucidated in our group [4], and it has been reported that HypF catalyses similar reactions [5]. Because of its close relationship to the domains of TobZ and HypF, it is likely that YrdC catalyses an adenylation reaction to form threonylcarbamoyl adenylate as precursor molecule for t^6A37 biosynthesis.

Here, we present the crystal structure of YrdC from *Methanocaldococcus jannaschii* with bound ATP. The structure shows a concave groove revealing the ATP binding pocket. It is formed by the characteristic KxRx(n)SxN motif of the YrdC/Sua5 family to bind to the phosphate moieties. From comparison with homologous structures an additional threonine binding cavity located near the ATP can be deduced. In the present structure a phosphate ion occupies this cavity mimicking the carboxyl group of threonine. Structure analysis further suggests an inversion of the -phosphate of ATP during the adenylation reaction catalysed by YrdC forming a threonylcarbamoyl adenylate.

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L29

THE FINE-TUNED MACHINERY OF O_2-TOLERANT [NiFe] HYDROGENASE

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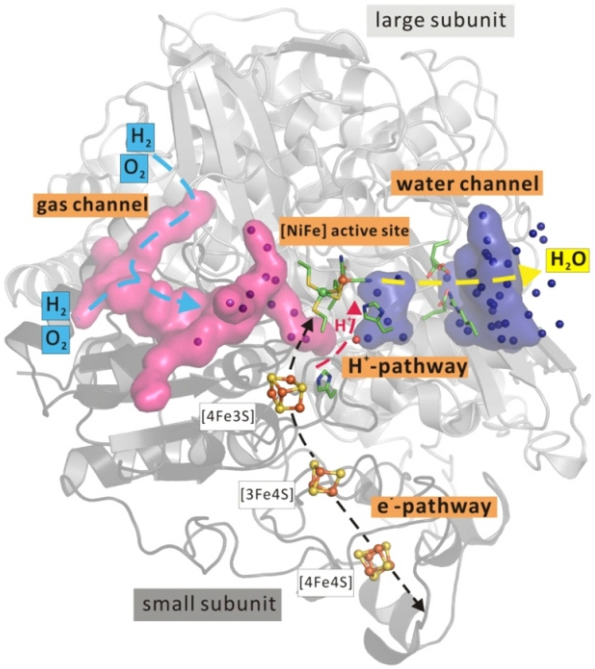
Hydrogenases are metalloenzymes catalyzing the heterolytic splitting of hydrogen into protons and electrons. In all three domains of life hydrogenases are domiciled, but only a small subgroup of [NiFe] hydrogenases evolved the feature of hydrogen conversion under aerobic conditions. For enabling the aerobic hydrogen oxidation in [NiFe] hydrogenases, multiple adaptable pathways have been evolved. Structural investigations on this biological machine might lead to new developments in the field of renewable energy technologies [1].

The membrane-bound [NiFe] hydrogenase (MBH) of *Ralstonia eutropha (R.e.)* is one of the best investigated typical O_2-tolerant hydrogenases. Several crystal structures of the MBH *R.e.* as wildtype or with multiple substitutions in different redox states reveal a highly fine-tuned interplay between pathways and channels that lead to a perfect transport of reagents and products to and from the active site [2, 3]. For hydrogen splitting the [NiFe] active site of MBH *R.e.* requires the delivery of hydrogen via a hydrophobic gas channel. Hydrogen oxidation liberates electrons which are guided via an electron pathway to an electron acceptor. Subsequently, the electrons enter the quinone pool of the respiratory chain as reduction power for the cell [2]. Under aerobic conditions additionally the [NiFe] active site has to reduce the attacking oxygen to water with 4 e^- and 4 H^+. On that account the electron pathway, consisting of three [FeS] clusters, has to operate bidirectional. A unique [4Fe3S] cluster proximal to the active site is mainly involved in this switch. This [4Fe-3S] cluster undergoes redox-dependent reversible transformations, namely iron-swapping between a sulfide, a peptide amide N. For proton delivery several pathways close to the active site have been investigated and introduce new questions that might be answered by investigative methods e.g. neutron diffraction. The gas channel that is supplying also the inhibitory oxygen has been adapted especially in quantity and size to remain the hydrogenase activity for the system [4]. Water molecules produced under oxygen reduction are released through a new water channel. This complex system is still not completely understood and moreover sensitive to X-rays. Consequently a near radiation-damage free technique, the free-electron laser (e.g. LCLS, Stanford, USA), has been used to gain further insights into the functionality of this enzyme.



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XPLORING THE STRUCTURAL MECHANISMS OF UBIQUITINATION REACTIONS

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A novice to the HEC community, we would like to introduce the Lorenz laboratory, our overarching research interests, and ongoing studies on the structural mechanisms of ubiquitination enzymes.

Ubiquitination is an extremely versatile post-translational modification that controls a wide variety of cellular pathways. It is accomplished through the sequential action of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). How ubiquitination enzymes achieve specificity in ubiquitin signaling is incompletely understood on a structural level.

Studies in our laboratory aim to unravel (i) how E2 and E3 enzymes achieve linkage specificity in ubiquitin chain formation, (ii) how E3 enzymes recognize and modify specific target proteins, and (iii) how the activities of E2 and E3 enzymes are regulated.

To answer these questions we employ X-ray crystallography and NMR spectroscopy, together with computational tools, biophysical measurements, and functional assays.

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L31

EXPLORING UNUSUAL MYXOBACTERIAL PATHWAYS-INSIGHTS INTO THE REGULATION OF ALTERNATIVE ISOVALERYL-CoA BIOSYNTHESIS

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Myxobacteria are of great biotechnological interest due to their usage of unusual biochemical steps during the production of a huge diversity of bioactive secondary metabolites. A number of these metabolites are produced from the common precursor isovaleryl coenzyme A (IV-CoA), which is normally derived from the degradation of leucine. Facing harsh conditions or leucine starvation an alternative pathway branching from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is used. It includes four chemical steps catalysed by five proteins, which are located on two different operons [1]. The expression of one of these operons is controlled by a transcriptional regulator, called AibR [2].

Here we present the crystal structure of AibR in the ligand free-state and bound to its possible regulator, IV-CoA. AibR shows the typical *TetR*-like transcriptional regulator architecture characterised by 9 -helices arranged in an N-terminal DNA-binding domain (helix-turn-helix motif) and a C-terminal ligand -binding domain (Fig. 1). By using microscale thermophoresis we could show the specificity towards IV-CoA and determined the K_D to 2 μM. Electrophoretic mobility shift assays revealed first insights into the mode of action of AibR mediated transcriptional regulation. From our results AibR seems to function in an unusual way to enable or block the transcription of the *aib*-operon.

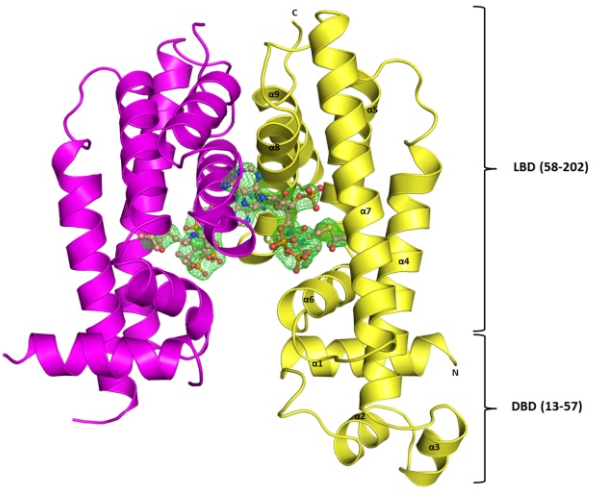

Figure 1. Structure of AibR in complex with IV-CoA. The difference electron density (F_O-F_C) is shown in green. DBD: DNA-binding domain; LBD: ligand-binding domain.

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STRUCTURAL AND MECHANISTIC ANALYSIS OF THE Slx1-Slx4 ENDONUCLEASE

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Holliday Junctions (HJs) are four way double stranded DNA structures appearing as intermediates in eukaryotic homologous recombination. Resolution of HJs is facilitated either by helicase/topoisomerase BTR complex or by structure specific endonucleases. Slx1 is one of the structure specific endonuclease belonging to GIY-YIG superfamily. Slx1 in complex with Slx4 acts upon varied DNA substrates. Here, we present a 2.1Å crystal structure of Slx1 from *C. glabrata* displaying a catalytic, N-terminal URI domain containing GIY-YIG motif and a C-terminal Zinc finger motif in a typical RING topology. While, Slx1 by itself exists as catalytically inactive homodimer, a complex of Slx1 with C-terminal conserved domain of Slx4 ($Slx4^{CCD}$) was found to be heterodimeric and catalytically active. Therefore, we propose a switch from catalytically inactive Slx1 homodimeric state to catalytically active Slx1-$Slx4^{CCD}$ complex as a regulatory mechanism for Slx1 endonuclease activity.