also a potent inhibitor of (Zn/Zn)bLAP. This combined approach provided insights on interaction of bLAP with sulphydryl-containing compounds, showing that metal exchange in site 1 modulates binding to these molecules that, depending on metal nature, may result as enzyme substrates or inhibitors.

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Keywords: biocrystallography of protein, proteins-inhibitor complexes, metalloenzymes

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The Crystal Structure of Human CA II Bound to a Strong Benzenesulfonamide Inhibitor

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes, which catalyze the reversible hydration of CO_2. In previous studies we have investigated by means of X-ray crystallography the rational design of sulfonamide/sulfamate/sulfamide inhibitors of this enzyme, which is involved in a multitude of physiological and pathological processes [1]. At least fourteen different CA isozymes are presently known in humans, and many of them are targets for the design of inhibitors with potential use as antiglaucoma, anti-obesity, or anticancer drugs among others. A class of CA inhibitors which showed very promising applications are the thioureas obtained from isothiocyanato sulfonamides and amines, hydrazines or amino acids. Such compounds generally showed potent inhibitory activity against the human cytosolic isozyme CA II as well as the transmembrane, tumor-associated isozyme CA IX, being thus interesting candidates for developing antiglaucoma/antitumor therapies based on them.

Here we report the first X-ray crystal structure of a thioureido-benzensulfonamide derivative in complex with human CA II as well as its inhibitory properties against isozymes I, II and IX [2].

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Keywords: biocrystallography of protein, protein-inhibitor complexes, rational inhibitor design

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Role of the Non-protein Ligand at the Ni-Fe Active Site of [NiFe] Hydrogenase

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Hydrogenases catalyze oxidoreduction of molecular hydrogen and have a potential value for a use of dihydrogen as an energy source.

[NiFe] hydrogenase possesses two oxidized states, Ni-A (inactive) and Ni-B (active). The pure Ni-A state was successfully prepared from the solution of the as-purified enzyme (mixture of the Ni-A and Ni-B states), and the crystal structures of both the Ni-A and Ni-B states have been determined at ultra-high resolution. The shape and size of the electron densities show that Ni-B possesses a monatomic non-protein bridging ligand between the Ni and Fe atoms at the active site and the cysteine sulfur ligand (Cys546) was modified by unknown atomic species (X546). Whereas Ni-A has a diatomic ligand at the bridging site and two systein sulfur ligands (Cys546 and Cys84) were also modified by unknown species (X546 and X84). X546 of Ni-A was shifted towards the Ni atom about 1.0 Å compaired to that of Ni-B. Diatomic bridging ligand and X84 of Ni-A seem to block the pathway of dihydrogen.

The essential features of the enzyme structure at the resting state and the transition mechanism from Ni-B to Ni-A are proposed.

Keywords: [NiFe] hydrogenase, Ni-A and Ni-B, non-protein ligand

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Crystal Structure of Mouse Carnosinase CN2 at 1.8 Å Resolution

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L-Carnosine, β-alanyl L-histidine, is found as a bioactive dipeptide which affects autonomic neurotransmission and blood pressure through histamenergic nerves and is present in mammalian tissues including the central nervous system. In mammals, two types of carnosinases, CN1 and CN2, both of which catalyse the hydrolysis of L-carnosine, with different properties are known. The mouse carnosinase CN2 was found to be highly concentrated in the parafascicular nucleus of the thalamus and so on in the brain, which suggests carnosine is degraded by CN2 to supply the substrate of histamine-synthesizing enzyme, histidine decarboxylase. We started crystallographic study of CN2 from mice to understand its enzyme mechanisms on a structural basis.

The MAD data were collected on beamline BL6A of the Photon Factory using an ADSC Quantum 4D CCD detector. The protein phases were determined with the program Sharp and improved with the program dm using non-crystallographic symmetry. The peptide model was built with the program ARP/wARP. The structure is now being refined with the program Refmac5.

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Keywords: metalloproteinases, enzyme active site, protein structure determination

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Class III Superoxide Reductase from *Treponema pallidum*

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Superoxide reductase of *Treponema pallidum* (*Tp* SOR) is a metalloprotein responsible for the scavenging of superoxide radicals in the cell [1]. SORs can be divided into three classes according to amino acid sequence alignment: Members of class I have only the catalytic domain. Class II and III SORs present an additional N-terminal domain that, in the case of class II, has an additional non-heme iron center ($Fe(Cys)_4$) of the rubredoxin type [2]. The active site, common to all three classes, is an iron center, ($Fe(Cys)(His)_4$) that reacts with superoxide in the reduced state.

*Tp*SOR is the first member of class III to be structurally characterized. Blue crystals of the oxidized form diffracted beyond 1.55 Å. A highly redundant in-house data set allowed solving the structure and synchrotron data led to phase improvement.

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Keywords: superoxide, soft X-rays, iron

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Crystal Structures of Cytochrome c Peroxidases from *Ps. nautica* and *Ps. stutzeri*

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Cytochrome c peroxidase (CCP) catalyses the reduction of H_2O_2 to H_2O, an important step in the cellular detoxification process. The structure of the di-heme CCP from *Pseudomonas nautica* 617 was obtained in two different conformations, which require calcium activation, correlated with a spin state transition of the peroxidatic heme. Form IN, oxidized, obtained at pH 4 does not contain Ca^{2+}. This inactive form presents a closed conformation the peroxidatic heme adopts a six ligand coordination, hindering the peroxidatic reaction from taking place. Form OUT, Ca^{2+} dependent, was obtained at pH 5.3, it shows an open conformation with the release of the distal histidine (His71) ligand, providing peroxide access to the active site. This form shows a bound Ca^{2+} ion, which is essential for the enzymatic activation, showing several conformational changes [1]. The structure of the CCP from *Ps. stutzeri* reveals a very similar conformation to the form IN of *Ps. nautica* 617. These structures provide us with some more clues about the role of the Ca^{2+} in the activation of CCP [2].

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Keywords: peroxidases, heme, calcium activation

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Structures of Matrix Metalloproteinase - 9 in Complex with Pharmacological Inhibitors

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The matrix metalloproteinases (MMPs) constitute a family of zinc endopeptidases with a metzincin-like catalytic domain [1]. They are involved in tissue remodelling, extracellular matrix degradation and futher biological processes. Under healthy conditions, their proteolytic activity is mainly regulated by the endogeneous tissue inhibitors of metalloproteinases (TIMPs). Disruption of this MMP-TIMP balance results in pathologies such as rheumatoid arthritis and osteoarthritis, atherosclerosis, heart failure, fibrosis, tumor growth and metastasis. MMP-9 is a key enzyme in the pathogenesis of heart failure and cancer [2]. MMP-9 activity could have an impact on the ventricular remodeling following infarction as well as in the blockage of tumor growth. Because the inhibition of MMPs is a promising approach for treatment of those diseases, synthetic MMP-9 inhibitors are developed as potential therapeutic agents for structure-based drug design.

We will describe high resolution crystallographic structures of the mutant (E402->Q) of the catalytic domain of MMP-9 with different synthetic inhibitors. One is based on pyrimidine-2,4,6-trione (RO-206-02222), the second on phosphinic acid (AM-409), the third on propionic acid (R1) and the last one is hydroxamic asid based on (MS-560). All of them possess high affinity towards MMP-9.

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Keywords: matrix metalloproteinase 9 (MMP9), inhibitors, structures

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Ru(bpy)$_2$(mbpy)-Adx(1-108) Complex: Photoreduction and Crystal Structure

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Bovine adrenodoxin (Adx) is a [2Fe-2S] protein. The truncated form Adx(1-108) was covalently modified with the ruthenium(II) bipyridyl complex ((Ru(bpy)$_2$(mbpy)) to test the "shuttle" hypothesis of the electron transfer mechanism.

The crystal structure of the Ru(bpy)$_2$(mbpy)-Adx(1-108) complex was solved by molecular replacement at 1.5 Å resolution. Ru(bpy)$_2$(mbpy) is covalently bound to Adx(1-108) and exposed to solvent. Two chiral components of Ru(bpy)$_2$(mbpy) cause two alternative conformations of the side chain of Cys95 of Adx(1-108).

Activity assays suggest that labeling might affect intermolecular electron transfer between redox-protein partners. The dye-associated photoreduction and chemical reduction of Adx is accompanied by a two-electron transfer. However, spin quantification points out that just one of the two iron atoms of the reduced Adx is in the Fe^{2+}-state.

Adx(1-108) can be photoreduced *via* the ruthenium compound, as confirmed by EPR. Ru(bpy)$_2$(mbpy)-Adx(1-108) does not display new *g* values. The electron transfer rate depends on the concentration of the complex, indicating intermolecular transfer to take place. Extrapolation to Adx concentration of zero gives the intramolecular rate constant. Possible electron transfer pathways calculated based on the 3D-structure are in the physiological range and could be related to the calculated intramolecular rate constant.

Keywords: metallo-enzymes, electron transfer, crystal structure

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Molecular Basis of the Myomesin Dimerisation: Implications for the Sarcomeric Assembly of the M-band

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Myomesin is an essential component of the sarcomeric M-band expressed in every type of vertebrate striated muscle analyzed so far. It is composed of 7 Ig-like and 5 Fn(III) domains. A unique sequence at the N-terminal part anchors myosin, while the central Fn(III) domains interact with the M4 domain of titin and the muscle-type creatine Kinase. These features favour specific lattice orientations and models of the M-band. A consistently important characteristic of myomesin that recently has been reported is its dimerisation via domain 13. We determined the structure of domains 12 and 13 revealing an antiparallel orientation of domain 13. Both domains 12 and 13 are Ig-like of type I. They are connected through a 22-residue helix that orients them to an almost vertical position. The overall assembly was confirmed *in vitro* by small angle X-ray scattering. For the *in vivo* confirmation of the assembly, we used a novel protein-complementation method utilizing truncated YFP mutants fused either to the N- or C-terminus of the myomesin dimerisation domain. Reconstitution of the intrinsic YFP-fluorescence could only be observed for the antiparallel orientation of the myomesin dimers, whereas constructs fused only N-terminally to myomesin displayed no fluorescence signal.

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Keywords: myomesin, M-band, sarcomere

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Structural Studies on Titin and Titin Kinase's Downstream Signaling Pathway

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Titin is the third filament system in vertebrate striated muscles where it spans half of the sarcomere from the Z-disc to the M-line. The giant multi-domain protein titin consists of about three hundred domains, most of them immunoglobulin (Ig)-like domains and fibronectin III (FnIII) domains showing typical patterns assigned to specific regions in the sarcomere, interposed by unique sequences. One of these is a serine/threonine kinase domain, titin kinase [1],