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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],