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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],

located at the interface between A-band and M-line. It has been shown by Centner et al. [2] that MURF-1, a member of the RING finger proteins, binds to the two Ig-domains A168 and A169 in proximity to the kinase. Thus, its binding might be involved in the regulation of titin kinase. The structure of this tandem Ig domain has been solved. Ig domains, also in titin, are involved in many protein-protein interactions and this interconnects titin with other muscle proteins and pathways. Here, we will present new structures near titin kinase and from a downstream signaling pathway of titin kinase (Gautel et al., unpublished data).

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Keywords: muscle proteins, immunoglobulin structure, protein interaction

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Carving a Beanstalk: the Structure of ΔS2 from Human Myosin II

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S2 is the flexible coiled coil that connects light meromyosin to the N-terminal motor domain of myosin II. S2 interacts with other proteins of thick filament and can lead to fatal familial hypertrophy (FFH) when mutated. We have determined the structure of a 126-residue N-terminal fragment of S2 in two different crystal forms. The WT protein diffracted to 2.7 Å resolution in a $C222_1$ cell of a=40, b=46, c=373 Å. Cryo-protection was difficult and data could only be reduced in XDS. Phases were derived from 2-λ MAD data collected from a mercury derivative. Only SHELXD with SHARP generated interpretable electron density maps. The protein is a parallel dimeric coiled coil of 187 Å lying stretched out along the c-axis.

The FFH-associated E924K-mutant crystallised in P1 with a=40, b=42, c=98 Å; α=91, β=93, c=107°. Molecular replacement was not successful and crystals were highly radiation sensitive, giving non-traceable electron density maps when anomalous phasing from SeMet-labelled or heavy-atom-soaked crystals was employed. It was, however, possible to locate 4 mercury atoms from anomalous data. These co-ordinates together with the position of cysteine residues in the WT structure were used in a semi-brute-force approach to derive the relative orientation of two coiled coils in the asymmetric unit. The model was refined to 2.5 Å with R=27.3 and R_{free}=34.9 %. The two extended coiled coils run anti-parallel with neighbouring molecules lying head-to-tail such that they form quasi-endless filaments.

Keywords: coiled coil proteins, MAD, brute force

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Preliminary X-ray Analysis of RNA Oligomers Containing CUG Repeats

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Human genome contains so many different types of repetitive sequences. Some of them are tandem repeats of trinucleotides, and their unusual expansions cause genetic diseases including type 1 myotonic dystrophy (DM1) and Huntington's disease (HD). The unit sequence for DM1 is CTG in the 3'-untranslated region of the myotonic dystrophy protein kinase (DMPK) gene, and that for HD is CAG in the ORF of exon-1 of the HD gene.

The two complementary sequences may induce increase or decrease of the repeats during DNA replication or repair of DNA. The direct origin of DM1 is, however, the transcribed RNA fragments with CUG repeats, which forms a specific structure and inhibits other protein syntheses. In the present study, structural versatility of such DNA and RNA fragments has been examined.

In the case of $(CUG)_n$, native PAGEs show that the even repeat (n=even number) is more stable than the odd repeat. This may be ascribed to the structural difference at the hairpin head. The PAGEs also suggest that duplex formation is dependent on coexisting cationic species and their concentration. Crystal data of $(CUG)_6$ are *a*=*b*=39.6 and *c*=141.0Å, space group *R*32 and one oligomer in the asymmetric unit. An approximate crystal structure has been solved by molecular replacement techniques at 1.9 Å resolution and shows that the fragment forms a duplex similar to an A-form RNA.

Keywords: RNA structure, X-ray analysis, genetic disease

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Crystal Structures of Two Minor Groove Binders Complexed with d(CGCGAATTCGCG)$_2$

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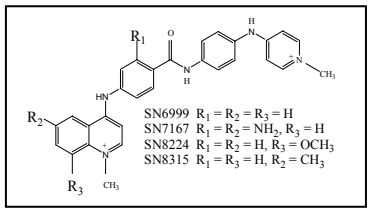
Quinolinium quaternary salts (QQS) are anticancer drugs [1] and bind reversibly in the minor groove of AT rich sites in DNA. The crystal structures of SN6999 [2] and SN7167 [3] complexed with CGC[e^{6}G]AATTCGCG and CGCGAATTCGCG respectively have been solved previously at a resolution of about 2.5 Å. We have recently solved the structures of two new QQS compounds, SN8224 and SN8315, complexed with the dodecamer CGCGAATTCGCG. These two structures are at higher resolution (1.6 and 1.8 Å) and crystallise in similar conditions. We are able to compare the four complex structures and reach conclusions about minor groove requirements for QQS compounds bound to unmodified DNA.



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Keywords: DNA, minor groove binders, anticancer drugs

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A new Highly Symmetric DNA G-4 Quadruplex/ Drug Structure

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Guanine-rich DNA telomeres occur at the 3' ends of chromosomes. They can associate into four-stranded assemblies known as stacked G-4 quadruplexes. It has been found that the enzyme telomerase protects tumour cells but not normal cells from telomere loss during replication. Telomerase has therefore become an exciting new target for anti-cancer drug design. Small molecules which can stabilise the formation of G-4 quadruplexes may inhibit telomerase activity.

We recently determined the crystal structure of the daunomycin complex with the telomeric sequence d(TGGGGT) [1]. We now report the 1.08 Å structure of daunomycin complexed to d(GGGG). The crystals are tetragonal, space group I4, a = b = 40.21, c = 49.83 Å. The final R is 16.1%. The asymmetric unit contains 2 independent strands of d(GGGG), 4 drug molecules, and eight Na and 2 Mg cations. The crystallographic 4-fold axis generates the biological unit which consists of 2 high-symmetry G-4 quadruplexes between which are 4 layers of daunomycin molecules with 4 daunomycins per layer.