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Iron-Sulfur Cofactor Assembly: the unique contribution of NMR

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Iron-sulfur (Fe/S) clusters are ubiquitous and evolutionary ancient prosthetic groups required to sustain fundamental life processes. The formation of intracellular Fe/S clusters does not occur spontaneously but requires complex biosynthetic machineries. Both the high complexity of these cofactors and their potential cellular toxicity, provided by the intrinsic redox chemistry of iron, cause that cells develop Fe/S protein assembly machineries comprising more than 30 proteins that synthetize Fe/S clusters and insert them in recipient apo-proteins (final targets) in a specific manner in different cellular compartments. Important mechanistic questions related to Fe/S cluster biosynthesis involve the molecular details of how Fe/S clusters are assembled on scaffold proteins, how [Fe-S] clusters are transferred from scaffolds to target proteins, how various accessory proteins participate in [Fe-S] protein maturation, and how the biosynthetic process is regulated. In this context, NMR is a precious tool of investigation because provides unique information fundamental to unravel such complex molecular mechanisms as well as electronic structure of Fe/S clusters. Fe/S protein maturation pathways occurring in different cellular compartments of human cells will be here described at the molecular level. The resulting picture moves forward the existing functional information on the protein components of these machineries.

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Unraveling the function of human BOLA proteins employing a CRISPR-Cas9-based tissue culture model

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Iron-sulfur (Fe/S) clusters are simple and evolutionary primordial inorganic cofactors, which are commonly coordinated by cysteine or histidine residues of proteins. Biogenesis of Fe/S proteins is rather complex, where the mitochondria-comprised ISC (iron sulfur cluster) assembly machinery plays the foremost role initiating this essential biosynthetic process. This work is primarily focused on the final steps of human mitochondrial [4Fe-4S] protein assembly including complex Fe/S proteins such as lipoate synthase (LIAS) and succinate dehydrogenase (complex II, SDH) containing 2 and 3 Fe/S clusters, respectively. This final step involves six known ISC proteins termed ISCA1, ISCA2, IBA57, NFU1, BOLA3 and IND1 1, 2. BOLA3 belongs to a family of characteristically three members, two of which are residing in mitochondria (BOLA1 and BOLA3) and the third one is cytosolic (BOLA2) 1.

BOLA3 was suggested as an ISC assembly factor, since patients harboring a frame-shift mutation in the BOLA3 gene (MMDS2 disease) presented with biochemical phenotypes encompassing impaired function of respiratory complexes I and II as well as of LIAS 1, 3. Since mutations in NFU1 showed similar phenotypes, it was suggested that BOLA3 plays a role in Fe/S protein metabolism, yet its precise molecular function was not revealed. The exact function of BOLA1 is unclear, yet ablation of BOLA1 in cultured human cells was found to increase mitochondrial protein thiol oxidation and cause alterations in mitochondrial morphology 4. An in vitro study has recently shown BOLA2 to be involved in the GLRX3-directed maturation process of Anamorsin (human DRE2 or CIAPIN1) 5. Whether BOLA2 plays a general role in cytosolic Fe/S protein maturation is currently unknown.

The aim of this project is to better define functions of the three BOLA homologs. We employed the CRISPR-Cas9 technology to delete the mitochondrial BOLA3 gene. As a result, we achieved a substantial drop in both the PDH and KGDH steady state levels. Additionally, we also found a reduction in SDH activity along with disruption of complex I subunits. These phenotypes match the MMDS2 disease situation but interestingly did not lead to a growth arrest of the cultured cells. Moreover, the BOLA3+BOLA1 double deletion mutant cells display aggravation of above-mentioned phenotypes although single deletion of BOLA1 was without severe consequences on Fe/S proteins. In all cases, the activity of the mitochondrial aconitase remained unchanged showing the striking target specificity of the mitochondrial BOLA proteins. Preliminary results show that deletion of BOLA2 did not result in any significant phenotype, e.g., leaving the cytosolic aconitase activity of IRP1 unaltered.

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In cellulo Mössbauer spectroscopy

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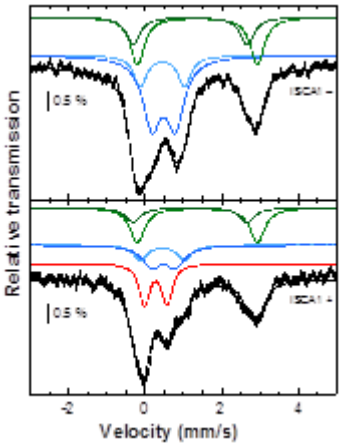
A-type proteins are essential proteins in the late stage of Iron-Sulfur Cluster (ISC) assembly1. Indeed it has been previously demonstrated that ISCA1 and ISCA2 are critical partners for the generation of Fe4S4 in the mitochondria of mammals2. To understand the intricate machinery of ISC assembly in mammals, we developed in vivo investigations based on Mössbauer spectroscopy, an appropriate tool to probe iron. This technic is specific to the 57Fe isotope and detects all 57Fe nuclei that are present in the sample. The signatures depend on the oxidation and spin states and on the chemical environments of the Fe ions. This presentation will focus on Mössbauer spectroscopy performed on cells where the murine ISCA1 and ISCA2 proteins have been expressed or not (see Figure). We will discuss the abundance and the nuclearity of the FeS clusters that are detected. This study complements the recent reports on in vitro NMR studies of human ICSA1 and ISCA23.



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The functional role of Glutaredoxin-3/BolA2 interaction in humans: an atomic level investigation

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Monothiol glutaredoxins (GRXs) are ubiquitous [2Fe-2S]-binding protein. In humans, a single domain GRX (human GRX5) is localized in the mitochondria, where it plays an important role in Fe-S clusters transfer, while a second, multi-domain GRX (human GRX3) is localized in the cytosol, where it likely has a role in cytosolic iron trafficking and cellular iron homeostasis, both functions requiring the binding of [2Fe-2S] clusters (1). Monothiol GRXs from several species have been found to interact in vivo with a second widely conserved protein family, named BOLA (2). In humans there are 2 mitochondrial BOLA proteins, BOLA1 and BOLA3, and one cytosolic BOLA protein, BOLA2. In yeast, monothiol GRX3-like cytosolic glutaredoxins form [2Fe-2S]-bridged hetero-dimers with the cytosolic BOLA-like protein Fra2 (3), and genetic studies demonstrate that the GRX-BOLA interaction is required for the efficient iron-dependent inhibition of the Aft1 and Aft2 activators, which are responsible for the transcription of iron uptake and storage genes (4). Recently it has been shown that, similarly to the yeast homologues, also the two tandem GRX-like domains of human GRX3 are able to form [2Fe-2S]-bridged complexes with human BOLA2 (5), but their role(s) have not been elucidated yet. We characterized in vitro the structural and metal binding properties of the human GRX3-BOLA2 hetero-complex, and investigated its potential functional role in transferring [2Fe-2S] clusters, by studying the interaction of the complex with a GRX3 physiological partner, the [2Fe-2S] cluster-binding protein Anamorsin, which is involved in the maturation of cytosolic Fe/S cluster proteins

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Function and evolution of mitochondrial Hsp70 system involved in FeS biogenesis.

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The mitochondrial pathway of FeS biogenesis was inherited from bacteria, including the involvement of Hsp70 chaperone machinery in the process. However, our evolutionary analysis revealed that while mitochondria did inherit the J-protein co-chaperone HscB, called Jac1 in Saccharomyces cerevisiae, from bacterial ancestors, it did not inherit the specialized Hsp70 HscA. In most species, including fungi distantly related to S. cerevisiae, plants, animals and humans, the major mtHsp70 functions in the FeS biogenesis pathway to transfer FeS from the scaffold on which they are built onto recipient proteins.

Available biochemical data indicate that the transfer of a FeS requires a typical Hsp70 reaction cycle. The specialized J-protein HscB/Jac1 binds a FeS scaffold using its C-terminal domain. Next, HscB/Jac1 interacts with Hsp70 via the N-terminal J-domain, which is highly similar to the J-domains of other J-proteins. This results in stimulation of Hsp70's ATPase activity that promotes its interaction with FeS scaffold and transfer of the cluster.

The system described above functions in most eukaryotes. However, S. cerevisiae and closely related fungal species express an additional mtHsp70, called Ssq1, which is specialized in FeS biogenesis. Similarly to multifunctional mtHsp70, it functions with Jac1, but in contrast to mtHsp70, its client binding specificity is restricted to the FeS scaffold, Isu. We demonstrated that Ssq1 evolved via an ancestral gene duplication of mtHsp70. One copy, called Ssc1 in S. cerevisiae, maintained biochemical properties typical for ancestral mtHsp70. The other copy diverged functionally to become Ssq1. In post-duplication species Jac1 coevolved with Ssq1, acquiring structural changes within its J-domain in the process. The altered J-domain became highly specific for Ssq1. Thus, Ssq1 and Jac1 form a highly specialized Hsp70 machine dedicated solely to Fe-S biogenesis. However, all evidence to date indicates that the mode of action of this newly evolved machine is the same as the one utilizing by multifunctional mtHsp70.

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Biochemical characterization of a novel radical-SAM enzyme with antiviral activity in humans

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Structure-function relationships of Protein Fe-S clusters assembly and degradation.

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Cluster assembly: The 2.74 Å resolution structure of th recombinantly produced (IscU-Ala35-IscS)2 complex from Archeoglobus fulgidus revealed that the IscS active site Cys321-containing loop is ordered and that the active cysteine thiol group approaches the [2Fe-2S] cluster assembled in IscU (1). Based on this observation we have proposed a plausible [2Fe-2S] cluster assembly mechanism, that involves direct S donation to the iron ions by the IscS active site persulfided cysteine.
Cluster degradation: The structure of the dimeric holo Fumarate and Nitrate Reduction regulator (FNR) from Aliivibrio fischeri has been solved at 2.65 Å resolution (2). FNR globally controls the transition between anaerobic and aerobic respiration in facultative anaerobes through its oxygen-sensitive [4Fe-4S] cluster. In the absence of O2, holo-FNR forms a dimer and specifically binds to DNA whereas in its presence the cluster is degraded causing FNR monomerization and DNA dissociation. We have used our crystal structure and the data from numerous FNR variants to propose that this process is controlled by extremely fine-tuned interactions, mediated by two salt bridges near the N-terminal cluster-binding domain and an "imperfect" dimer coiled coil interface. [4Fe-4S] to [2Fe-2S] cluster degradation propagates a conformational signal that indirectly causes monomerization by disrupting the first of these interactions and unleashing the "unzipping" of the FNR dimer in the direction of the C-terminal DNA-binding domain.

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The roles of ferredoxin:NADP(+) oxidoreductase in photosynthetic energy conversion

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Plant photosynthetic linear electron transport chain (LET) transfers electrons generated by photosystems II and I to a small iron-sulphur protein ferredoxin (Fd). Fd acts simultaneously as a bottleneck and as a branching point that distributes high-energy electrons to several alternative electron transport chains and a multitude of enzymes. However, the dominant pathway in chloroplasts is the one that produces NADPH, by the activity of ferredoxin-NADP+-oxidoreductase (FNR). FNR is a soluble protein that exists in several isoforms and utilizes two reduced Fds to produce one molecule of NADPH. We have demonstrated that thylakoid rhodanase-like protein TROL acts as a bona fide photosynthetic membrane attachment point for FNR1, although other associations with thylakoid and inner envelope membranes have been described. We have also proposed a scheme for the dynamic FNR recruitment to TROL2,3. We speculate that TROL-FNR interaction represents branching point between electron-conserving and electron-dissipating pathways. Alternative Fd-dependent pathways downstream of PSI and different from the LET may become dominant by the dynamic detachment of FNR form TROL, thus suggesting a novel mechanism of photosynthesis regulation. Also, efficient scavenging of O2.- radicals by FNR-dependent mechanism can be envisaged.

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A structural biology perspective on key iron sulfur cluster assembly pathways in mitochondria

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Mitochondria contain the complex Iron-Sulfur-Cluster Assembly (ISC) machinery, which is crucial for the maturation of both mitochondrial and cytosolic iron-sulfur (Fe/S) proteins in eukaryotes.1
Two major iron sulfur cluster assembly processes in mitochondria are essential for cell life. The first assembles the [2Fe-2S] cluster and subsequently the late acting machinery assembles the [4Fe-4S] clusters. Here we presenting some results on both complex assembly processes obtained using several techniques.
In vivo synthesis of [2Fe–2S] clusters on the mitochondrial scaffold protein Isu1 requires the cysteine desulphurase complex Nfs1-Isd11, frataxin, ferredoxin Yah1 and its reductase Arh1. We reconstitute [2Fe–2S] cluster synthesis on Isu1 in a reaction depending on Nfs1-Isd11, frataxin, Yah1, Arh1 and NADPH. NMR spectroscopy, combined with other techniques such like MicroScale Thermophoresis and CD, helps to find out that only reduced Yah1 tightly interacts with apo-Isu1. NMR structural studies identify the Yah1–apo-Isu1 interaction surface and suggest a pathway for electron flow from reduced ferredoxin to Isu1.2
Lately in the assembly machinery, the generation of [4Fe-4S] clusters critically depends on two proteins named ISCA1 and ISCA2 which perform a non-redundant functional role forming in vivo a heterocomplex. In this work we have structurally characterized the Fe/S cluster binding properties of human ISCA2 and investigated in vitro (using NMR, EPR, and UV-vis) whether and how a [4Fe-4S] cluster is assembled when human ISCA1 and ISCA2 interact with the physiological [2Fe-2S]2+ cluster-donor human GRX5.3

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Iron-sulphur clusters in DNA replication and repair

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In recent years, multiple proteins essential for DNA replication and repair have been shown to require binding to an iron-sulphur (FeS) cluster for their function. The presence of these cofactors in proteins involved in DNA metabolism is surprising, given that – upon FeS cluster oxidation – free iron atoms may generate DNA damaging hydroxyl radicals via Fenton chemistry. At the same time, their redox sensitivity makes FeS clusters particularly interesting and versatile cofactors that would be uniquely suited e.g. to sense oxidative stress and to allow adaption to suboptimal conditions of DNA replication. However, very few studies have addressed these possibilities so far.
We are using a combination of techniques, such as biochemistry/biophysics and molecular/cellular biology, in order to understand the function of FeS clusters in proteins involved in DNA replication and repair. In particular, we focus on the DNA helicases ChlR1 and DNA2, as well as the replication enzyme DNA polymerase delta. A second line of research deals with the maturation of nuclear FeS proteins by the cytoplasmic iron-sulphur assembly (CIA) targeting complex.

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The Monothiol Glutaredoxin GrxD is Essential for Growth and Iron Homeostasis in Aspergillus fumigatus

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Efficient adaptation to iron starvation is an essential virulence determinant of the most common airborne fungal pathogen Aspergillus fumigatus. In the current study we characterized the role of the A. fumigatus monothiol glutaredoxin ortholog GrxD (Afu2g14960), orthologs of which mediate cellular transport and sensing of iron in S. cerevisiae and S. pombe.
Heterokaryon rescue technology proved that grxD is an essential gene in A. fumigatus, which contrasts S. cerevisiae and S. pombe. Conditional expression of grxD demonstrated that GrxD deficiency can be partially compensated by high iron supplementation. Taken together with the transcriptional upregulation of grxD during iron starvation, these data are in agreement with a role of GrxD in cellular iron transport. In a shift from GrxD-inducing to -repressing conditions under iron starvation, the grxDc strain displayed increased accumulation of protoporphyrine IX (PpIX), the iron-free precursor of heme. This is consistent with a role of GrxD in iron sensing as PpIX accumulation is a hallmark of deficiency in HapX, the major transcription factor required for adaptation to iron starvation. Moreover, we identified a strain, which carries a grxDc suppressor mutation enabling growth in standard medium. Transcriptional analysis combined with genetic mapping revealed that the suppressor phenotype is caused by derepression of iron uptake via inactivation of sreA, which encodes a transcriptional repressor of siderophore biosynthesis and reductive iron assimilation.

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MICROSECOND TIME SCALE PRE-STEADY-STATE KINETICS OF METALLOPROTEINS

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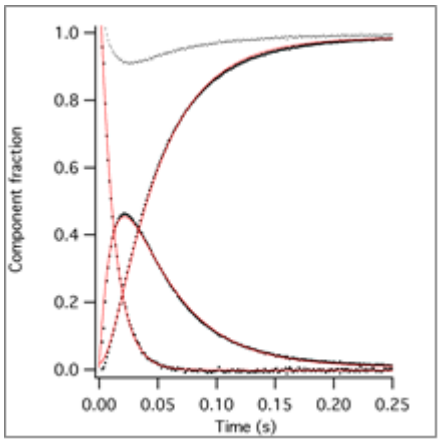
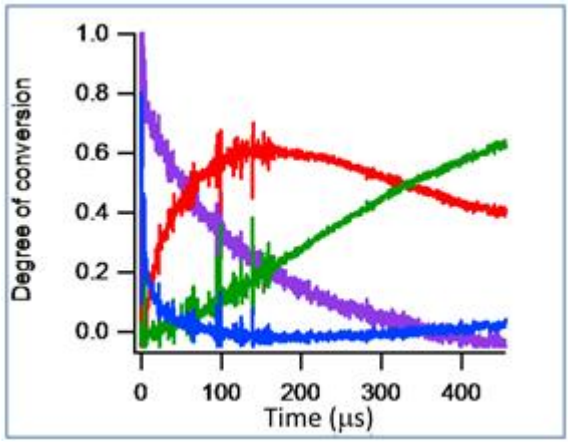
Experimental studies to develop molecular mechanistic models of protein action require the time resolution of a single full turnover and the spectroscopic identification of each protein intermediate involved. The color and magnetism associated with transition ions frequently provide a convenient handle to monitor intermediates of metalloproteins by means of optical or EPR spectroscopy. However, the encountered kcat's are of the order of up to 107 s-1, and this dictates challenging technological constraints on the design of mixing and sampling devices.

With the main objective of reducing the typical dead time of a few milliseconds of spectrokinetic instrumentation to (sub)microseconds a long-term research effort was run in Delft by Simon de Vries until his unexpected passing away last year. De Vries was, for example, well on his way in developing practical analysis procedures, based on the principle of 'singular value decomposition', to extract spectra of multiple metalloprotein intermediates occuring at short time scales, and he was close to completing the construction of an optical continuous-flow UV-vis spectrometer with a dead time of a few microseconds.

The present authors have taken it upon them to safeguard the scientific heritage of de Vries and to further develop and apply his methods and instrumentation for the pre-steady-state kinetic analysis of metalloproteins with maximal time resolution. We will illustrate our recent efforts on two examples: (i) the re-evaluation of the reaction mechanism of the calcium-PQQ enzyme glucose dehydrogenase from Acinetobacter calcoaceticus (left figure); and (ii) the discovery of an early new phase in the re-folding of acid-unfolded horse cytochrome c (right figure).





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Mutant ISCU and the Muscle-specific Phenotype

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Hereditary myopathy with lactic acidosis (HML) is an autosomal recessive disease caused by an intronic mutation in the iron-sulfur cluster assembly (ISCU) gene, resulting in aberrant splicing (1, 2) resulting in a non-functional ISCU protein. This leads to defects in several Fe-S containing proteins in the respiratory chain and the TCA cycle. The symptoms in HML are restricted to skeletal muscle, and it has been shown that this is due to higher levels of incorrectly spliced ISCU in skeletal muscle compared with other energy-demanding tissues (3). The splicing of ISCU most likely involves a collection of splicing factors where each participant plays a specific role. We have previously identified PTBP1, IGF2BP1 and Caper as modulators of the aberrant ISCU splicing, using an ISCU mini-gene in human RD4 cells (4). PTBP1 was shown to repress the incorrect splicing while IGF2BP1 and Caper enhanced the incorrect splicing. IGF2BP1 is particularly interesting because it shows a higher affinity for the mutant ISCU sequence, however, even though it binds RNA it has no known splicing activity, and how it contributes enhanced incorrect splicing is not known. Using a transgenic mouse model, we could confirm that the aberrant splicing of mutant ISCU is more pronounced in muscle compared to other tissues, with slow-fiber muscle showing the highest levels of incorrectly spliced mutant ISCU. We have identified a factor that might be involved in this muscle-type specific splicing of ISCU.

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On the way to the cytosol: Iron-sulfur cluster assembly in trypanosomes

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We studied members of the export and cytosolic CIA machineries in Trypanosoma brucei, an excavate parasitic protist. ABCB transporter TbAtm was involved in Fe-S export from the mitochondrion unlike the other transporter TbMdl, which was playing role in heme homeostasis. Sulfhydryl oxidase TbErv1 showed its presence in the mitochondrial intermembrane space. There it plays a role in the transport of small sulfur containing proteins into the mitochondrial inner membrane space and its involvement in the Fe-S pathway seems to be rather indirect. It works most likely as a solo player, since we did not detect a TbMia40 homologue or other obvious interaction partner by tandem affinity purification and mass spectrometry. Further we focused on proteins that are involved in the final step of the CIA pathway, in which the Fe-S cluster is transferred onto the Fe-S apoproteins. All identified components were cytosolic, while TbCia2B containing a Fe-S cluster assembly domain was also present in the nucleus. The CIA-targeting complex is essential for the procyclic and bloodstream stages of T. brucei and its components display novel regulatory roles on the mitochondrial and cytosolic aconitase. A cryo-entrapment supported pull-down of the CIA components identified three distinct complexes formed by TbCia1-TbCia2B, TbCia1-TbMms19-TbCia2B and TbCia1-TbCia2A, and also showed the mutually exclusive binding of TbCia1 with TbCia2A and TbCia2B. Combined, our data provided insights into the structure of iron-sulfur cluster assembly in a highly divergent eukaryote, and identified its novel regulatory features.

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Electrochemistry for the functional analysis of FeS proteins and enzymes

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Electron transfer between proteins and electrodes can be either direct or mediated, and achieved in a variety of configurations: with the protein and/or the mediator free to diffuse in solution, immobilized in a thick, hydrated polymer film, or adsorbed as a sub-monolayer on the electrode. The experiments can be performed with the goal to study the protein or to use it. Mechanistic studies are easier in the configuration where the protein is adsorbed and electron transfer is direct. In the case of redox proteins, information can be obtained about the thermodynamic properties of the centers (reduction potential, pKa), and the kinetics of electron transfer and of coupled reactions (such as proton transfer). In the case of large redox enzymes, direct electrochemistry measures how the turnover rate depends on experimental parameters (electrode potential, pH, T, substrate and inhibitor concentrations, light…) and gives information about various steps in the catalytic mechanism.1-6 However, not all proteins and enzymes can be addressed using direct electrochemistry.

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Understanding the cellular pathway of Iron Sulfur cluster proteins”

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Iron-sulfur (Fe/S) clusters belong to the most ancient co-factors of proteins involved in electron transfer, catalysis and DNA interaction and maintainers processes. The different kind of Fe/S clusters, [2Fe-2S] and [4Fe-4S] types, contain iron in a ferrous (Fe2+) or ferric (Fe3+) state and sulfide (S2-) and are usually integrated into proteins via coordination bond between the iron ions and a cysteine or histidine residues. It is now clear that several rare human diseases are attributable to defects in the process of Fe/S cluster biogenesis. Cytosolic monothiol glutaredoxins are implicated in eukaryotic cells in intracellular iron trafficking and sensing via their bound [2Fe-2S] clusters. Experimental evidences define a novel role for the human cytosolic monothiol glutaredoxin 3 (GRX3) as a cluster transfer protein. A cytosolic partner of GRX3, is the electron transfer protein Anamorsin, that contain two [2Fe-2S] clusters.
We expressed and characterize this two proteins to study the protein-protein interaction and the cluster transfer between, to rebuild the maturation pathway of anamorsin.
Understanding the different interactions, helps us to understand which mechanisms are faulty in pathological situations.

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NEET PROTEINS – A UNIQUE CLASS of 2Fe-2S PROTEINS INVOLVED IN HEALTH AND DISEASES

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The NEET family was descovered in 2007 in human to be comprised of three different proteins encoded by CISD1, CISD2 and CISD3 genes. MitoNEET (mNT:CISD1) and NAF-1 (Miner1:CISD2) the two major members of the newly discovered NEET class of 2Fe-2S proteins, are both situated in the outer mitochondrial membrane (OMM), while NAF-1 is also situated on the outer ER and ER-Mitochondrial associated (MAM) membranes. mNT and NAF-1 were shown to be involved in a diverse array of biological processes including autophagy, apoptosis, aging, diabetes, cancer and iron and reactive oxygen (ROS) homeostasis. The mNT and NAF-1 proteins are endowed with the 'NEET fold' that is comprised of a homodimeric intertwinned structure containing two major domains; a β-cap domaIin and a cluster binding domain. Each monomer, in mNT and NAF-1, contains a [2Fe-2S] cluster that is coordinated by the "finger-print"cluster-ligation of the NEETs' [2Fe-2S] clusters. The latter is composed of three Cys and one His (3Cys:1His) residues.This coordinating groups render the clusters with high stability, yet, confirms them with a unique lability that probably results from the HIS being on the NEET proteins' surface exposed to protonation. Those properties were demonstrated experimentally as well as the cluster-transfer from native NEETs to apo-acceptor protein(s) in solution or to mitochondria in permeabilized cells (1). Abrogation of the labile features is attained by mutating the His (87 in mNT and 114 in NAF-1) to Cys as well as by pretreatment of the NEET proteins with different drugs (e.g. pioglitazone). The drugs were shown to bind to the His (and other amino acids) and to stabelize the [2Fe-2S] cluster. Recently, structural and functional characterization (in knockdown and RNAi plant line) of Arabidopsis NEET revealed an ancient role for NEET proteins in iron metabolism (1). Moreover, the availability of the three NEET structures enabled the finding that despite their high structural similarity, the electrostatic surface and hydrophobic regions are different for each NEET protein, suggesting different protein partner(s). Recently, we found that the NEET proteins interact with key proteins involved in apoptosis and mitochondrial metabolism; While NAF-1 interacts with Bcl-2/BCl-XL and iASPP, mNT interacts with VDAC. These interactions alter the stability of the NEET [2Fe-2S] cluster and its ability to be transferred to acceptor protein(s) or mitochondria. Our recent in vivo studies demonstrated that NAF-1 and mNT, play a major role in epithelial breast cancer cells. NAF-1 that was shown to be a key player in regulating autophagy and mNT was proposed to mediate iron and reactive oxygen homeostasis in mitochondria. We showed that the protein levels of NAF-1 and mNT are elevated in human epithelial breast cancer cells, and that suppressing the level of these proteins using shRNA results in a significant reduction in cell proliferation and tumor growth, a decrease in mitochondrial performance, uncontrolled accumulation of iron and reactive oxygen in mitochondria, and activation of autophagy. In our very recent study we we reported that overexpression of NAF-1 in xenograft breast cancer tumors results in a dramatic augmentation in tumor size and aggressiveness, and that NAF-1 overexpression enhances the tolerance of cancer cells to oxidative stress. Remarkably, overexpression of a NAF-1 mutant with a single point mutation that stabilizes the NAF-1 cluster, NAF-1(H114C), in xenograft breast cancer tumors results in a dramatic decrease in tumor size that is accompanied by enhanced mitochondrial iron and reactive oxygen accumulation and reduced cellular tolerance to oxidative stress. Furthermore, treating breast cancer cells with pioglitazone that stabilizes the 3Cys-1His cluster of NAF-1, results in a similar effect on mitochondrial iron and ROS accumulation. Taken together, our findings point to a key role for the unique 3Cys-1His cluster of NAF-1 in promoting rapid tumor growth through cellular resistance to oxidative stress. Cluster transfer reactions mediated by the

overexpressed NAF-1 protein are therefore critical for inducing oxidative stress tolerance in cancer cells leading to rapid tumor growth, and drugs which stabilize the NAF-1 cluster could be used as part of a treatment strategy for cancers that display high NAF-1 expression. We are also involved in studing the genetic disorder Wolfram Syndrome type 2 (WFS-2) where the NAF-1 protein is missing. WFS-2 is an inherited multi-organ disorder that appears to be abundant in our regional local populations. WFS-2 characterized by optical nerve atrophy, deafness and □-cell dysfunction results from cellular stress and apoptosis leading to sever insulin deficiency causing diabetes type I like phenotype. WFS-2 is a disease caused by a missense mutation in the CISD2/NAF-1 gene that leads to the absence of expression of a [2Fe-2S] protein the NAF-1. Our recent studies of both fibroblast cells obtained from paitents and of a model sysytem of Insulinoma □-cells (INS-1E) has shown that some of the pathophysiological disorders resulting from NAF-1 suppressed expression and/or genetic absence can be compensated by treatments with a combination of chelating agent that removes access of iron and reducing agent that treats the accumulation of ROS.

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Analysis of protein complexes necessary for efficient FeS cluster formation on Isu1 protein

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Maturation of iron-sulfur proteins involves complex biosynthetic machinery. Specific interactions among various components of the mitochondrial ISC system are necessary for formation of FeS cluster on the molecular scaffold – Isu1 protein and for the cluster transfer to the appropriate acceptor protein. In vivo synthesis of [2Fe-2S] clusters on the mitochondrial scaffold protein requires the cysteine desulfurase complex Nfs1-Isd11, Yfh1 (yeast homolog of human frataxin), ferredoxin Yah1 and its reductase Arh1.

The main purpose of my study is to reconstitute FeS on Isu1 in vitro and to verify what is the effect of abnormal protein:protein interactions crucial for reconstitution of FeS within the molecular scaffold on the kinetics of this process. In vitro reconstitution of FeS clusters on proteins can be monitored using circular dichroism spectroscopy (Webert et al., 2014 Nat Commun. 5: 5013). This method requires a highly concentrated molecular scaffold protein, for this purpose we decided to use Isu1 protein from thermophilic fungus – Chaetomium thermophilum.

Preliminary in vitro experiments showed that the purified Isu1 protein from C. thermophilum interacts with components of the ISC system from Saccharomyces cerevisiae: cysteine desulfurase (Nfs1/Isd11) and yeast frataxin Yfh1 protein. In addition, we showed that C. thermophilum Isu1 interacts with yeast chaperones - Ssq1 and Jac1. Most importantly, the interaction with chaperones is functional because C. thermophilum Isu1 stimulates Ssq1's ATPase activity in the presence of co-chaperones: Jac1 and Mge1.

Next using CD spectroscopy to monitor in a real time assembly of FeS cluster on Isu1 protein in the presence of purified components of the ISC machinery we found that Yfh1 variants with W131A and D86KE89K mutations, defective in interaction with Isu1 protein and cysteine desulferase respectively, have much reduced activity upon reconstitution of FeS on Isu1. Similarly, an Isu1 variant with alanine substitutions within the PVK motif, and thus defective in Yfh1 binding, was also defective in FeS assembly. Together, these results confirm the importance of Yfh1 interactions with other components of the assembly complex for efficient FeS cluster assembly.

We also found that this new reconstitution system did not support FeS assembly on the Isu1-D77A variant, although the FeS cluster was readily assembled on it in the presence of DTT. Because homologous D/A variants were commonly used in previous structural and functional studies as stable substitutes for native FeS-Isu complexes, our observations raise the question whether the Isu1-D77A variant serves as a scaffold or target during FeS cluster assembly.

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Composition of the CIA targeting complex and binding to FeS proteins

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Iron-sulfur (FeS) clusters are inorganic cofactors found in a wide variety of proteins carrying out fundamental biological processes, such as electron transfer (e.g. in respiratory complexes I, II, III), catalysis (mitochondrial aconitase), and cellular iron homeostasis (IRP1).
During the past years many proteins involved in essential steps of DNA replication and repair were also found to coordinate an FeS cluster (e.g. Pol α, Pol δ, Pol ε, DNA2, XPD, FANCJ, RTEL, ChlR1). This finding was rather surprising, since FeS clusters may generate reactive oxygen species upon oxidation and potentially damage DNA.
The maturation of all nuclear FeS cluster proteins depends on the iron-sulfur cluster (ISC) assembly machinery in mitochondria. From there an unknown iron compound is exported to the cytoplasm, which is required for the assembly of an FeS cluster on a scaffold complex. The incorporation of a fully assembled FeS cluster into an FeS apo-protein is carried out by the late components of the cytosolic iron-sulfur cluster assembly machinery (CIA), namely MMS19, MIP18 and CIAO1, that together form the so-called CIA targeting complex. However, not much is known about the process during which an FeS cluster is transferred to an FeS apo-protein.
We investigated the composition of the CIA targeting complex and its interaction with client Fe-S proteins. We determined the interaction sites of MIP18 and CIAO1 with MMS19 and analysed which CIA machinery partners are required for the interaction with client FeS proteins. Finally, our data suggest a tight regulation of MIP18 protein levels that is dependent on the direct interaction of MIP18 with MMS19.

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Mammalian Iron-Sulfur Cluster Biosynthesis: The possible role of frataxin

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Iron-sulfur (Fe-S) cluster-containing proteins are essential components of cells. In eukaryotes, Fe-S clusters are synthesized by the mitochondrial iron-sulfur cluster (ISC) machinery and the cytosolic iron-sulfur assembly (CIA) system. In the mammalian ISC machinery, pre-assembly of Fe-S cluster on the scaffold protein (ISCU) involves a cysteine desulfurase complex (NFS1/ISD11) and frataxin (FXN), the protein deficient in Friedreich's ataxia. By comparing biochemical and spectroscopic properties of quaternary (ISCU/NFS1/ISD11/FXN) and ternary (ISCU/NFS1/ISD11) complexes, we have shown that FXN stabilizes the quaternary complex and controls iron entry to the complex through activation of cysteine desulfurization. Furthermore, we have shown that under these conditions a Fe-S cluster is formed within the quaternary complex that can be transferred to mammalian aconitase (mACO2) to generate an active enzyme. Together, these data help further unravel the Fe-S cluster assembly process and the molecular basis of Friedreich's ataxia

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Mass spectrometric studies of metallochaperones

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Trafficking of essential metal ions like copper, iron and zinc in cellular cytoplasm and in various organelles like mitochondria is often assisted by various metallochaperones, which could be defined as proteins specified for delivery of metal ions to target proteins. Well known are copper chaperones which assure safe handling and specific delivery of potentially harmful copper ions to cellular copper proteins like Cu-ATP-ases, Cu,Zn-superoxide dismutase and cytochrome c oxidase. Zinc ions are stored and delivered to different zinc enzymes and zinc finger proteins by metallothioneins. Synthesis and delivery of iron-sulphur clusters in mitochondria is accomplished by so called iron-sulphur cluster assembly or scaffold proteins, which could also be defined as iron chaperones. Mass spectrometry (MS) is a well-suited technique for studies of metal-binding stoichiometry, metal-binding mechanism as well as metal-binding affinity of different metallochaperones and corresponding examples are presented. By using MS metal-binding stoichiometry as well as mechanism for binding of Zn(II) and Cd(II) ions to metallothionein-1 and -3 has been determined (1). Analysis of the metal-binding affinities of various copper chaperones and their partner proteins by MS allowed us to establish affinity gradients determining copper transport in the cell (2). MS has also been used for determination of the stoichiometry of Fe-S cluster in ISCA2 protein and studies of the cluster stability towards oxidants and dithiothreitol (3).

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The Orange Complex from Sulphate Reducing Bacteria

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Cells division mechanism has been mostly studied in Escherichia coli, Bacillus subtilis and Caulobacter crescentus. Recently, a protein complex was proposed to be involved in cell division of anaerobic microorganisms.[1] In Desulfovibrio vulgaris Hildenborough (DvH), this complex is composed by DVU2103, DVU2104, DVU2105 and DVU2109, in addition to the Orange Protein (ORP) (DVU2108), several of which are annotated as metalloproteins. Expression of the genes encoding these proteins is regulated by a □54-dependent transcriptional regulator, DVU2106.[1] The ORP has been previously isolated from D. gigas as a soluble monomeric protein with an unique mixed metal sulfur cluster [S2MoS2CuS2MoS2]3- and the apo-ORP can be reconstituted either as a Mo-Cu or W-Cu cluster.[2,3,4]

Here, the homologous expression of DVU2103 encoding a Fe-S cluster ATPase has shown that it co-purifies with ORP. DVU2103 presents a broad absorption band at 400 nm, characteristic of [4Fe-4S] cluster and a molar extinction coefficient per protein of ε400 nm = 32600 M-1 cm-1, with a reduction potential of around -400 mV. This, together with its EPR spectra, suggests the presence of two [4Fe-4S] clusters, which are oxygen sensitive. In addition, the ORP from DvH was heterologously produced and biochemically characterized. UV-visible titrations using (NH4)2MoS4 and CuCl2, in the presence and absence of apo-ORP, have shown that it favors an 2Mo:1Cu stoichiometry and that the metal cluster synthesis is assisted by the protein.[5] The DVU2109 homologue in D. alaskensis G20, Dde3202, was also biochemically characterized as a Fe-S protein.

Preliminary data indicates that the ORP system might in fact be involved in Fe-S biosynthesis or repair, with a secondary effect in cell division.We thank Fundação para a Ciência e Tecnologia for the financial support to SRP(FCT-ANR/BBB-MET/0023/2012), and Lab-RMN at FCT-UNL and Rede Nacional de RMN for access to the facilities.

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Carbonylation of HSA leads to disruption of metal ion-protein interaction

Ana Penezic

Metal ions have pivotal roles as enzyme cofactors in oxygen transport and redox processes in living systems. HSA is a metal ion transporter having one high-affinity binding site for copper(II) ions located on the N-terminus. Having in mind that unbound copper(II) ions can undergo Fenton/Haaber Weiss reaction leading to free radical production, a decrease in HSA copper binding affinity/capacity would contribute to the development of oxidative stress.

In order to investigate this phenomenon, we took CD and fluorescence emission spectra of commercial HSA (essentially copper free as control), HSA-Cu (II) complex, carbonylated commercial HSA (HSA-MG) and carbonylated HSA-Cu (II) complex. Carbonylation of samples was monitored by measuring the content of Cys34 thiol via Ellman's test. All samples were also analysed by SDS and native electrophoresis. Copper-(II) ion release was measured by spectrophotometrically, using bathocuproine as chromogen.

CD spectra show no significant differences in secondary structure between carbonylated and copper loaded HSA. Fluorescent spectra, on the other hand, showed significant differences in intrinsic emission originating from Trp214 (43% reduction in carbonylated sample). Electrophoretic mobility of carbonylated samples in native gels changed, as well as the intensity of bands originating from copper loaded samples, while SDS gels showed existence of additional band in lanes containing carbonylated samples. The content of copper-(II) ions in modified samples was lower as well as the content of Cys34 thiol groups.

Our results show that carbonylation of HSA-Cys34 thiol group leads to reduction in copper binding affinity.

Cytosolic and nuclear iron-sulfur proteins and their assembly

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Up to 50 cytosolic and nuclear Fe/S proteins act in a wide range of cellular processes in eukaryotes. These proteins are of key importance for ribosomal maturation1, tRNA modification2, DNA replication3 and repair, and are thus found in all eukaryotes. Insertion of the Fe/S clusters is carried out by the socalled cytosolic iron sulfur protein assembly (CIA) machinery composed of 11 proteins. Determinants for recognition of apo-Fe/S proteins among the ~10,000 cytosolic/nuclear proteins, which a typical eukaryote contains, remain completely obscure. A path driven by cluster transfer to a thermodynamically favoured binding site at the target is tacitly assumed, similar to the belief in a spontaneous path before discovery of Fe/S biosynthetic machineries. This "thermodynamic model" has been challenged by various observations, suggesting a selective protein-guided process. Several factors of the ISC machinery have specific target apo-Fe/S proteins, a LYR primary sequence motif is a determinant for mitochondrial maturation and different affinities for target Fe/S proteins were detected by mass spectrometric analysis of late CIA components. Activity measurements of intrinsic and ectopic enzymes in mutants are used in my lab to identify amino acid sequence motifs for CIA-dependent maturation. In Patras I will report on the coordination and cluster type of Dre2, an early CIA factor4.

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Using mouse and cellular models to understand the pathophysiology underlying Fe-S cluster biogenesis deficit

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Friedreich's ataxia (FRDA), the most common autosomal recessive ataxia, is characterized by a sensory and spinocerebellar ataxia, hypertrophic cardiomyopathy and increase incidence of diabetes. FRDA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. Impaired mitochondrial oxidative phosphorylation, bioenergetics imbalance, deficit of Fe-S cluster enzymes and mitochondrial iron overload occur in individuals with FRDA. To date, no treatment exists for stopping or slowing FRDA disease.
Over the past years, we have generated cellular and mouse models that reproduce important progressive pathological and biochemical features of the human disease, including cardiac hypertrophy, mixed cerebellar and sensory ataxia, Fe-S enzyme deficiency, and intramitochondrial iron accumulation. These models have enabled us to demonstrate that Fe-S deficit is a primary event of the disease leading to iron metabolism deregulation through the activation of the iron-regulatory protein, IRP1. These models are excellent models for deciphering the physiopathology of the disease and for testing pre-clinical therapeutic protocols. The latest advances in understanding the pathophysiology will be discussed, with a particular emphasis on new neurological models that are being developed as well as therapeutic approaches.

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[FeFe] hydrogenases as studied by EPR, vibrational Spectroscopy and electrochemistry; The quest for the catalytic mechanism

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[FeFe] hydrogenases are the fastest enzymes in H2 production and oxidation. Their extraordinary activity is related to the unique composition of the active cofactor, a [2Fe] unit linked through a cysteine thiol group to a "classical" [4Fe-4S] cubane sub-cluster. The two iron centers in the binuclear co-factor are kept in a low oxidation state, Fe(I) or Fe(II), through the coordination of two CN and three CO donor ligands as well as a bridging aza-dithiolate (ADT) ligand. The distal iron Fed has an open coordination site where presumably the hydrogen substrates (H+ or H2) bind and are converted. The pendant amine group in the ADT bridge functions as proton shuttle toward this exchangeable site. The heterolytic splitting mechanism of H2 requires a hydride intermediate. Using the recently developed artificial maturation technique,1-2 we were able to insert an artificial 57Fe labeled [2Fe] sub-site featuring an oxa-dithiol (ODT) bridging ligand into HydA1, the [FeFe] hydrogenase from Chlamydomonas reinhardtii. Using Nuclear Resonance Vibrational Spectroscopy (NRVS) we detected the unique vibrational signature of an iron bound terminal hydride. This hydride state is stabilized because the proton channel has been disrupted. Furthermore, using pH dependent FTIR spectroelectrochemistry of WT HydA1, we identified two singly reduced states of the H-cluster with different electronic configuration depending on the protonation state suggesting "internal proton coupled electron transfer" (PCET). These two observations lead us to suggest an ECEC sequence for the catalytic mechanism.

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Deciphering the late-steps of FeS cluster assembly in plant organelles

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In plants, iron-sulfur (Fe-S) proteins are involved in crucial processes such as photosynthesis or respiration to cite only a few. The Fe-S proteins are first synthesized as apoproteins and the prosthetic groups are inserted into the polypeptide through dedicated assembly machineries. Plants have three Fe-S cluster assembly machineries, namely SUF, ISC and CIA, devoted to the maturation of plastidial, mitochondrial and nuclear or cytosolic proteins, respectively. Genetic studies indicate that these machineries are essential but the precise molecular mechanisms controlling the late steps of this maturation process, in particular the trafficking of Fe-S clusters achieved by transfer proteins, are still insufficiently characterized. By combining biochemical, structural and spectroscopic approaches together with genetic and physiological approaches in Arabidopsis thaliana, we aim at deciphering the roles of organellar transfer proteins of the glutaredoxins, BolA, NFU and ISCA families.

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ENHANCEMENT OF STABILITY OF SULFUR CONTAINING PROTEINS BY COVALENT IMMOBILIZATION ON POLYMERS

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Nowadays, a great deal of attention has been paid to natural enzymes, as they show advanced catalytic features, being, therefore, involved in various field, as follows: biochemistry, food and chemical industry, agriculture and medicine. However, their short catalytic lifetimes hinder their applicability. Thus, enzyme immobilization emerges as a means of overcoming the above mentioned drawback, several approaches being considered in this respect: adsorption, entrapment, microencapsulation or covalent bonding.

Among enzymes, polyphenol oxidase (PPO), has been given increased importance as it plays a key role, both in plants and animals, i.e. it is responsible for biosynthesis of melanin in animals and browning in plants. Its isoelectric point corresponds to a pH of 4.5. This enzyme is a dinuclear copper-containing one consisting of various amino acids. Histidine (His) is the major amino acid found in PPO. Small amounts of Cysteine (Cys), a sulfur-containing amino-acid, may be found, as well. Therefore, it may be assumed that PPO is a sulfur-containing enzyme. As copper is present within the enzyme as well, PPO could be categorized as a sulfur-copper enzyme, belonging to the larger class of sulfur-metals proteins, that includes also sulfur-iron proteins.

There is a wide range of materials worthy to be considered as supports for enzyme immobilization, both inorganic and organic. Polymers used as supports becomes more and more important due to several advantages: various classes of polymers available and appropriate, copolymers combining advantages of individual polymers can be obtained, and functional groups of polymers can be transformed in other groups more suitable for the immobilization.

Electro-conducting polymers, such as polypyrrole (PPY), exhibit enhanced chemical/thermal stability, alongside high conductivity and availability of preparation in a wide range of solvents.

Polymer membranes, exhibits several noteworthy features, including high specific surface, bio-catalysis and separation functions, are efficiently integrated in one structure.

The present work was aimed at investigating the enzyme immobilization process of PPO on two types of supports, i.e. PPY, an electroconducting polymer, and acrylic membranes. Both polymers involve firstly an activation stage with glutardialdehyde to insert binding sites for enzyme immobilization.

Raw materials and final products were characterized using various techniques: XPS, Raman spectrometry, XRD, SEM FTIR, TGA, DSC, AFM. The occurrence of important changes in chemical composition, thermal behavior and morphology, confirmed the success of both reactions, functionalization and immobilization, respectively. Moreover, when membranes were used, the catalytic activity of immobilized enzymes was shown to be preserved at elevated levels.

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Three-act story of labile iron pool: adrenaline, ascorbate, and amino acids

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Labile iron pool is composed of redox-active Fe complexes. They comprise a heterogeneous population of small metabolites/ligands, large poly-functional ligands (e.g. polypeptides), surface components of membranes and extracellular matrix, and organic anions. The involvement of poorly liganded iron in different pathologies, such as neurodegenerative or cardiovascular diseases, via Fenton reaction and other redox processes, has been widely recognized. Under physiological settings, ligands might modulate Fe transport and availability, whereas Fe appears to be involved in degradation of specific metabolites. However, the exact speciation, structural and redox properties of Fe-ligand complexes in the labile iron pool are still largely unknown.1

Our group is focused on the modulation of redox activity of iron by small extracellular metabolites/ligands. We are interested in: (i) the structure of Fe-ligand complexes; (ii) redox reactions between Fe, metabolites/ligands, and O2; and (iii) the impact of pro-oxidative activity of iron on biomolecules. The research primarily relies on EPR spectroscopy (low-T, spin-trapping), biochemical techniques (spectrophotometry, oximetry, assays), and cyclic voltammetry, but we also use NMR, Raman spectroscopy, HPLC, fluorometry, and molecular and cell culture methods. Here, we present examples of three intertwined components of our research.

Adrenaline forms 1:1 or 1:3 complexes with Fe3+. Altogether, 1:3 complex appears to be more stable, but at higher Fe3+ concentrations, 1:1 complex is energetically more favorable, probably due to entropy increase via water molecules release from [Fe(H2O)6]3+ complex. Adrenaline is stable in these complexes, i.e. there is no significant level of adrenaline oxidation/Fe3+ reduction. On the other hand, adrenaline acts as a catalyst of Fe2+ oxidation by O2. Superoxide radical anion and H2O2 are produced, whereas adrenaline might be degraded only via side-reactions (probably with hydroxyl radical (HO•)).2

Ascorbate complex with Fe3+ takes part in a two branch redox system. In the first branch, Fe3+ acts as a shuttle for 2e– transfer to O2 to produce H2O2. This reaction represents the basis of anticancer effects of ascorbate that have been observed in vitro, spurring the interest for the applicability of mega-doses of ascorbate in cancer treatment. However, in the second branch, 1e– transfer from ascorbate to ferric iron gives rise to Fe2+. It has been shown that the second branch is promoted with increasing iron concentrations. When iron is present at physiological concentrations, Fe2+ prevents H2O2 accumulation via Fenton reaction. Unlike H2O2, HO• has an extremely low diffusion radius and cannot enter and kill cancer cells.3

Amino acids react with HO• at different rates. We have examined the correlation between physicochemical properties of amino acids and their anti-HO• activity in the Fenton system. The activity showed a general positive correlation with hydrophobicity and negative correlation with polarity. Most importantly, HO•-provoked oxidation of amino acids was strongly affected by hydrophobic hydration.4 The movement of HO• in water involves hydrogen exchange chain-reaction with water molecules (HO• + H2O → H2O + HO•), which appears not to go through hydrogen bonds.5 Transfer of HO• from the bulk water to the water molecules in the hydrophobic hydration shell and further to an amino acid might be promoted by the lower density of hydrogen bonds down that route. Our findings imply that damaged proteins with exposed hydrophobic side-chains might act as sacrificial antioxidants.

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Biogenesis of iron-sulfur proteins in the cytosolic and nuclear compartment of eukaryotic cells

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Maturation of iron-sulfur (Fe/S) proteins in eukaryotic cells requires a compartmentalized set of three bioge¬nesis systems. Mitochondria not only harbor their own ISC assembly system but also support the biogenesis of extramitochondrial Fe/S proteins by releasing an ill-defined sulfur-containing compound (X-S) via the ISC ex¬port system. Fe-S protein maturation within the cytosol/nucleus is mediated by the CIA system and reminiscent of the ISC assembly process. Tracing extramitochondrial Fe/S protein assembly by 55-iron radiolabeling re¬vealed a staged sequence in which at an early phase a preliminary cluster is assembled on a Nbp35-Cfd1 scaffolding complex. This initial assembly step requires assistance by the Tah18-Dre2 (NDOR1-CIAPIN1) electron transport chain and by a glutaredoxin. Interaction studies suggest that subsequent cluster release from the scaffold involves the hydrogenase-related protein Nar1 (IOP1). Finally, dedicated CIA targeting com¬plexes mediate the specific insertion of Fe/S cofactors into cognate apo-proteins. The branching character of the CIA system becomes particularly evident by analysis of Fe/S protein assembly in mammalian cells. Nota¬bly, one major CIA pathway is established by CIA2A and especially supporting proteins involved in the regula¬tion of cellular iron homeostasis. The recently identified Lto1-Yae1 (ORAOV1-YAE1D1) adaptor complex is indispensable for Fe/S cofactor insertion into the ribosome recycling factor Rli1 (ABCE1) by docking to the Cia1-Cia2-Mms19 targeting complex, thereby highlighting the vital importance of Fe/S protein assembly.

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Resonance Raman spectroscopy of Fe-S proteins: from ferredoxins to DNA repair enzymes

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Resonance Raman spectroscopy (RR) provides sensitive and selective information on chromophores in proteins and enzymes. Upon excitation into S-to-Fe(III) charge transfer band, RR spectra of Fe-S containing proteins reveal vibrational fingerprint of the cluster, allowing for discrimination between different cluster types, and furthermore, between the vibrational modes of inorganic core and those that engage Cystein ligands. In the past 30 years, RR has been widely used for disentangling of structure-function relationship in Fe-S proteins and cellular processes that involve these proteins (e.g. biogenesis of the clusters).
We have employed RR to extract fine details about clusters in several studies, including identification of Fe-S clusters in respiratory chain complexes, detection of Fe- donor to the E. coli scaffold IscU1 and monitoring of thermally induced disassembly of Fe-S centers in feredoxin.2 Recently, we have studied a disputed role of the [4Fe-4S] cluster in DNA repair enzymes, using Endonuclease III (EndoIII) from D. radiodurans as a model.3 This DNA glycosylase belongs to the BER pathway that is conserved across all kingdom of life and represents a homologue of the central enzyme for repair of ROS damaged DNA in humans. RR revealed details on interaction of three different EndoIIIs with DNA substrates, while Surface enhanced RR (SERR) of the proteins attached to functionalized metal electrodes, allowed simultaneous probing of structural and redox properties of the cluster. These first ever reported SERR spectra of an Fe-S protein provided direct evidence that the [4Fe–4S] cluster is responsible for the redox activity of EndoIIIs. It was shown that this process is not exclusively DNA-mediated, as postulated, and that the proposed mechanism of DNA repair initiation needs to be revised.

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Redox players in mitochondria biogenesis and apoptosis

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Protein import into mitochondria is fundamental for their biogenesis and therefore critical for cell survival. Protein biogenesis in the intermembrane space relies on the Mia40 pathway that orchestrates oxidative folding of proteins in this mitochondrial compartment. The key components Mia40 and Erv1 of this pathway are known but regulation of this process and its putative links to redox homeostasis and redox signalling are still elusive. We find that (i) the Mia40 pathway is influenced by hydrogen peroxide (ii) a fraction of cytosolic redox active proteins like thioredoxin and the thiol peroxidase are targeted to the intermembrane space. The presence of these antioxidant machineries in the IMS ensures protection against oxidative stress damage in a compartment-specific manner. On the other hand, an atypical partner for Mia40, the human Fe/S protein
protein ciapin (which is localised primarily in the cytosol) functions as a metal stress sensor shifting between and anti-apoptotic and a pro-apoptotic role in response to iron levels in the cell. The ramifications of these unexpected protein targeting and protein interaction pawthays for cell physiology, apoptosis and survival will be discussed.

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DNA replication inhibitor hydroxyurea alters Fe-S centers by producing reactive oxygen species in vivo

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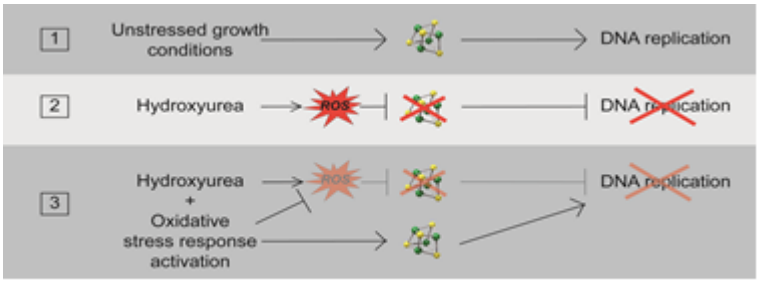
Redox homeostasis is tightly controlled in cells as it is critical for most cellular functions. Iron-Sulfur centers (Fe-S) are metallic cofactors with electronic properties that are associated with proteins and allow fine redox tuning. Following the observation that altered Fe-S biosynthesis is correlated with a high sensitivity to hydroxyurea (HU), a potent DNA replication blocking agent, we identified that oxidative stress response pathway under the control of the main regulator Yap1 attenuates HU deleterious effects, as it significantly increases resistance to HU, Fe-S biosynthesis and DNA replication kinetics in the presence of HU. Yap1 effect is mediated at least in part through up-regulation of two highly conserved genes controlling cytosolic Fe-S biosynthesis and oxidative stress, Dre2 and Tah18. We next observed that HU produces deleterious effects on cytosolic Fe-S clusters in proteins in vivo but not in vitro, suggesting that HU's impact on Fe-S in vivo is mediated by cellular metabolism. Finally, we evidenced that HU exposure was accompanied by production of reactive oxygen species intracellularly. Altogether, this study provides mechanistic insight on the initial observation that mutants with altered Fe-S biosynthesis are highly sensitive to HU and uncovers a novel mechanism of action of this widely used DNA replication inhibitor.



Model for DNA replication control by HU through Fe-S alteration.

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