

Pre steady-state kinetics of redox metalloenzymes using ultrafast mixing techniques

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Understanding the molecular mechanism of enzyme catalysis requires detailed structural and mechanistic information that can only be obtained through a combination of techniques and experiments. Pre-steady state, or rapid kinetics techniques, such as stopped-flow UV-vis absorbance or fluorescence spectroscopy are highly important tools to determine rate constants of individual steps in a catalytic mechanism, and to identify intermediates that will help us to understand the mechanism. The dead time of a conventional stopped flow instrument is approximately 1 ms, which does not allow the observation of intermediates with a shorter lifetime. By using a specially designed four-jet tangential micromixer we have been able to construct a **continuous flow UV-vis spectroscopic device, called the Nanospec**, with a dead time of 4 μ s. And, using the same mixer, a freeze quench instrument called the **Microsecond freeze Hyper Quenching device (MHQ)** with a dead time of 80 μ s. Cytochrome c refolding kinetics during a pH jump from 2 (unfolded) to 4.7 (near native state) was studied in the submillisecond timescale using two different rapid mixing techniques. The combination of the UV-vis and EPR data show that within 1 millisecond cytochrome c folding proceeds through three different steps: 1) His18 binding to the heme cofactor, 2) a conformational change that affects the heme environment, 3) Met80 binding to the heme cofactor in concert with a significant increase of the alpha helical content of the protein. There is evidence for further slower conformational steps towards the stable folded state at pH 4.7. Further applications with iron-sulfur cluster containing enzymes and proteins are presented.

Science Type / Oral or Poster: Oral

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Sensing of Fe-S clusters in proteins by mass spectrometry

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Keywords: MALDI MS, ESI MS, Fe-S clusters

Iron-sulphur (Fe-S) clusters represent a frequent cofactor in hundreds of proteins, which biological functions include electron transfer, redox and nonredox catalysis as well as stabilization of protein structure. It has been established, that formation of Fe-S clusters *in vivo* is not spontaneous process and therefore there exist also a number of scaffold and carrier proteins, which functions are connected to assembly and transfer of Fe-S clusters to the target proteins. Major Fe-S clusters contain $\text{Fe}^{2+/3+}$ and sulfide²⁻ in [2Fe-2S], [4Fe-4S] or [3Fe-4S] configurations, which are bound to proteins mainly via Cys but also by His, Asp or Ser residues. Although Fe-S proteins are attractive targets for MS studies, there are limited number of successful attempts to probe Fe-S clusters by MS. Surprisingly there are reports about detection of Fe-S clusters by MALDI MS but ESI MS studies prevail. There seems to be two major obstacles, on the MS studies of Fe-S cluster proteins. First, Fe-S clusters are sensitive to oxygen and it is difficult to run MS experiments in oxygen-free environment. Secondly, ESI is coupled to electrical potential, which might oxidize the samples in positive mode of measurements and lead to decomposition of clusters. By these and other reasons there are only handful papers dedicated to MS of Fe-S proteins, which are overviewed in the current presentation. Mammalian ferredoxins are known to contain [2Fe-2S] cluster, which participate in electron transfer. It is considered that Fe-S clusters in mammalian ferredoxins and in its yeast homologues are stable even in aerobic conditions, which is mainly confirmed by UV-VIS and EPR spectroscopy, however, despite of that there are no data about detection of Fe-S cluster in mammalian ferredoxins and its yeast homologues by ESI MS. Our attempts to detect Fe-S cluster in yeast adrenodoxin homolog 1 (Yah1), expressed recombinantly in *E.coli* and purified by IMAC were unsuccessful. ESI MS spectrum in standard conditions displayed metalloform with two Fe^{2+} ions as major peak, metalloform with two iron ions and two DTT molecules as minor peak and multiple apoprotein adducts with one, two and three S atoms. In the follow-up experiments systematical study on the effects of electrospray ionization parameters on the detection of Fe-S cluster in Yah1 was undertaken.

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Iron metabolism regulators: unraveling new targets of the IRP/IRE system

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Keywords: IRP, IRE, ppp1r1b, iron metabolism

Cellular iron homeostasis is tightly regulated by the iron regulatory proteins (IRPs) 1 and 2 that bind *cis*-regulatory iron-responsive elements (IRE) on target messenger RNAs (mRNA). Under iron deprivation conditions, IRPs bind to IRE sequences and regulate postranscriptionally gene expression. To expand the understanding of the IRE/IRP regulatory network, we have previously deciphered 35 novel mRNA that bind to both IRP1 and IRP2 from five different tissues in mice (Sanchez et al. 2011). Among the new targets, we have recently studied *profilin 2 (Pfn2)* mRNA and described its role in maintaining iron homeostasis in cell lines and mice (Luscieti et al. 2017). Now, as a novel IRP-interacting transcript, our work focuses on protein phosphatase 1 regulatory subunit 1B (Ppp1r1b) which acts as a phosphatase or kinase inhibitor depending on its own phosphorylation state. Interestingly, Ppp1r1b has been recently linked as a negative regulator of transferrin internalization (Collinet et al. 2010). Thus, we hypothesize that Ppp1r1b expression may be regulated in response to cellular iron levels. Our studies reveal that mouse Ppp1r1b mRNA contain an IRE in its 5' UTR that binds to recombinant IRP *in vitro*. Overexpression of Ppp1r1b in HeLa cells reduces its metabolically active iron pool (LIP, labile iron pool). Overall, our results indicate a plausible novel function of Ppp1r1b in iron homeostasis regulation.

Science Type / Oral or Poster: Oral

References: 1. M. Sanchez et al. Blood 118 (2011) e168-79. 2. S. Luscieti et al. Blood 130 (2017) 1934-45. 3. C. Collinet et al. Nature 464 (2010) 293-9. Funding: Work partially subsidized by projects SAF2015-70412-R by MINECO, CODYSAN by Ramon Areces Foundation, DJCLS R 14/04 - Deutsche Josep Carreras Leukämie-Stiftung and with financial support from Josep Carreras International Foundation and Obra Social "la Caixa" granted to MS.

De novo expression of transfected Sirtuin 3 enhances susceptibility of human MCF-7 breast cancer cells to hyperoxia treatment

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Keywords: sirtuin 3, MCF-7, hyperoxia, reactive oxygen species (ROS), mitochondrial function

Sirtuin 3 (Sirt3) has a promising role in cancer tumorigenesis and treatment, but there have been controversies about its role as oncogene or tumor suppressor in different types of cancer. Changes in its expression are associated with the excessive production of reactive oxygen species (ROS), thus contributing to mitochondrial dysfunction and age-related pathologies. Hyperoxic treatment (i.e. generator of ROS) was shown to support some tumorigenic properties, but finally suppresses growth of certain mammary carcinoma cells. Due to strikingly reduced Sirt3 level in many breast cancer cell lines, we aimed to clarify the effect of de novo Sirt3 expression upon hyperoxic treatment in the human MCF-7 breast cancer cells. De novo expression of Sirt3 decreased metabolic activity and cellular growth of MCF-7 cells, reduced expression of pro-angiogenic and epithelial mesenchymal transition genes, induced metabolic switch from glycolysis to oxidative phosphorylation, and decreased abundance of senescent cells. These effects were enhanced upon hyperoxic treatment: induction of DNA damage and upregulation of p53, with increase of ROS levels followed by mitochondrial and antioxidant dysfunction, resulted in additional reduction of metabolic activity and inhibition of cellular growth and survival. The mitigation of tumorigenic properties and enhancement of the susceptibility of the MCF-7 breast cancer cells to the hyperoxic treatment upon de novo Sirt3 expression, indicates that these factors, individually and in combination, should be further explored in vitro and particularly in vivo, as an adjuvant tumor therapy in breast cancer malignancies.

Science Type / Oral or Poster: Oral

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Comparative biochemical analysis of specialized and multifunctional Hsp70/Hsc20 systems functioning in iron-sulfur cluster (FeS) biogenesis

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Keywords: Hsp70, Iron–sulfur clusters, J-domain proteins

Hsp70/Hsc20 protein chaperones involved in FeS biogenesis have a complex evolutionary history. In bacteria, specialized Hsp70(HscA) functions with specialized J-protein co-chaperone Hsc20 (also called HscB). In mitochondria of most species, including humans, the homolog of Hsc20 functions with multifunctional mtHsp70, as Hsp70(HscA) was lost during evolution. As a result, mtHsp70 is involved in FeS biogenesis, as well as general protein folding and protein translocation. Late in fungal evolution, in an ancestor of *Saccharomyces cerevisiae*, a specialized Hsp70(Ssq1) emerged as a consequence of mtHsp70 gene duplication.

Studies from many laboratories have established that, whether a general or specialized Hsp70 is involved, chaperones play a critical role in FeS transfer from the scaffold protein Isu (IscU in bacteria) onto a recipient protein - via an ATP driven cycle of interaction between the scaffold and the Hsp70/Hsc20 system. Yet, since studies on each of the systems were carried out in different laboratories, much less is known how similar or different the systems are at the biochemical level.

We compared biochemical properties of the Isu/IscU binding cycles using purified proteins from *E. coli*, *S. cerevisiae* and, as representative of a species utilizing mtHsp70 for FeS biogenesis, the thermophilic fungi *Chaetomium thermophilum*. We found that specialized Hsp70(Ssq1) and bacterial Hsp70(HscA) converged biochemically, despite a different evolutionary origin. Both specialized Hsp70s have a similar affinity for Hsc20, form stable complexes with Isu/IscU in a Hsc20 dependent reaction, and their ATPase activities are stimulated by saturating concentrations of Hsc20 and Isu/IscU to similar maximal values. In contrast, multifunctional mtHsp70 has a markedly lower affinity toward Hsc20 and does not form a stable complex with Isu. Yet, its maximal ATPase activity, measured upon saturation with Hsc20 and Isu, is comparable with those of specialized Hsp70s. Our results suggest that the system based on the multifunctional mtHsp70 compensate for a lower affinity for Hsc20 by higher protein concentrations. Indeed, mtHsp70 is one of the most abundant mitochondrial proteins.

In sum, Hsp70s functioning in FeS biogenesis utilize two biochemically different strategies. One based on high affinity toward Hsc20 and Isu, and the other based on low affinity, but high protein abundance. It remains to be determined which of these strategies is more robust against perturbations.

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A mutation within the short presequence of ISCA1 causes multiple mitochondrial dysfunction syndrome 5 (MMDS5) with infantile-onset leukodystrophy and impaired mitochondrial [4Fe-4S] protein assembly

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Keywords: [Fe-S]-biogenesis, MMDS, lipoylation, respiration, aconitase

Multiple Mitochondrial Dysfunction Syndromes (MMDS) are characterized by infantile-onset mitochondrial encephalopathy, myopathy, non-ketotic hyperglycinemia, and lactic acidosis. Particularly the latter two are consequences of impaired respiratory chain activity and lipoic acid metabolism. The syndromes are caused by functional deficiencies of mitochondrial [4Fe-4S] protein assembly (ISC) components, leading to improper maturation of mitochondrial key Fe/S enzymes including respiratory chain complexes I to III, electron transfer protein dehydrogenase (ETFDH), and lipoic acid synthase (LIAS). Four different MMDS have been analyzed in detail according to the genes involved in the disease, MMDS1 (NFU1), MMDS2 (BOLA3), MMDS3 (IBA57), and MMDS4 (ISCA2). MMDS5 has recently been attributed to defects in the ISC component ISCA1, but a cellular analysis of the syndrome is lacking. Here, we biochemically characterise a MMDS5-causing homozygous ISCA1 p.V10G missense mutation within the mitochondrial presequence affecting both organellar import and protein stability. Down-regulation of ISCA1 in HeLa cells by RNAi impaired the biogenesis of multiple mitochondrial [4Fe-4S] proteins, yet could be complemented by expression of wild-type ISCA1. In contrast, the ISCA1 p.V10G mutant protein only partially complemented the defects and elicited MMDS5-related biochemical phenotypes even upon enhancement of mitochondrial import efficiency by a potent Su9 targeting sequence from *Neurospora crassa* subunit 9 of mitochondrial ATPase. Analysis of mitochondrial import revealed that the ISCA1 presequence is generally not cleaved upon organellar entry, indicating that the missense mutation remains part of the mutant protein and affects its function. Collectively, our comprehensive clinical and biochemical investigations show that the presequence of ISCA1 maintains a critical functional relevance even after mitochondrial import. The p.V10G mutation within the presequence impairs the activity of ISCA1, resulting in disturbed mitochondrial [4Fe-4S] protein maturation and causing the observed severe MMDS5 phenotype.

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Expression of Nitrogenase FeMo-co Biosynthetic Proteins in Eukaryotes

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Keywords: nitrogenase, eukaryotes, synthetic biology, SAM-radical

The generation of cereal plants able to express nitrogenase and thus being capable of utilizing atmospheric nitrogen would change agricultural systems worldwide. Nitrogenases are two-component metalloenzymes consisting of a dinitrogenase and a dinitrogenase reductase, which in the case of the most abundant and catalytically efficient molybdenum nitrogenase are called MoFe protein and Fe protein, respectively. Nitrogenases are found exclusively in some prokaryotes. Programs to engineer active nitrogenase in plants and other eukaryotes must deal with large number of genetic parts required for nitrogenase assembly and function, and also with nitrogenase sensitivity towards O₂. Nitrogenase genetic engineering can be accomplished out by putting together four distinct genetic modules: the Fe protein module, the MoFe protein module, the FeMo-cofactor biosynthetic module, and the electron transport module. Here we use yeast as model organism and a combination of synthetic biology and biochemical complementation assays to explore functionality of these modules in eukaryotes. Because of their central and unique roles, special attention is given to the NifH and NifB iron-sulfur proteins required for the biosynthesis of nitrogenase FeMo-cofactor. Progress towards expression of NifH and NifB in eukaryotes will be presented.

Science Type / Oral or Poster: Oral

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New antibacterial drugs against the [4Fe-4S] quinolinate synthase

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Keywords: 4Fe-4S enzyme, antibacterials, NAD, NadA

NAD (nicotinamide adenine dinucleotide) plays a crucial role in many essential biological reactions. Quinolinic acid is a precursor of this cofactor in most organisms, but its biosynthetic pathway differs among them. In most eukaryotes it is produced from the degradation of *L*-Tryptophan whereas in prokaryotes, it is synthesized from *L*-Aspartate and Dihydroxyacetone phosphate as the result of the concerted action of two enzymes: *L*-aspartate oxidase or NadB and Quinolinate synthase or NadA.^[1] Apart of these “de novo” pathways, most of the organisms count on a salvage pathway to synthesize NAD from small molecules such as nicotinamide or nicotinic acid. However, in some pathogens such as *Helicobacter pylori* and *Mycobacterium leprae*, responsible for gastric cancers and leprosis respectively, no salvage pathway exists and consequently, NadB and NadA constitute interesting antibacterial targets in these pathogens.

In the laboratory, we are interested in NadA enzyme which is a [4Fe-4S] enzyme. Its Fe-S cluster that is essential for its activity is coordinated by 3 cysteine ligands and a water molecule.^[2] The first *in vitro* and *in cellulo* inhibitor of NadA was described in our laboratory: the dithiohydroxyptalic acid or DTHPA (Figure 1) inhibits the activity of NadA enzyme by interaction with the cluster through its thiolate groups.^[3] However, a lack of selectivity was observed at high concentration. Based on a structure-activity study using DTHPA derivatives some new inhibitors have been designed (Figure 1). I will present their synthesis, their inhibitory effect on NadA enzyme *in vitro* as well as some spectroscopic and structural data which explain their mechanism of action on NadA.

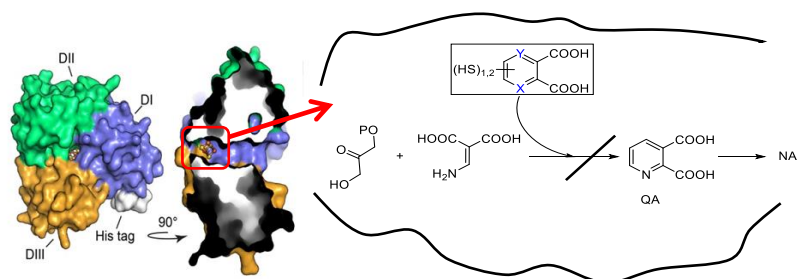


Figure 1: NadA structure showing the tunnel that connects the surface of the protein to the catalytic Fe-S cluster where condensation between Iminoaspartate and dihydroxyacetone occurs. DTHPA and some of its derivatives as potential better inhibitors of NadA are shown.

Science Type / Oral or Poster: Oral

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Dual localization of TROL: defining and substituting chloroplast import determinants

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Keywords: protein import, TROL, chloroplasts, localization

TROL (thylakoid rhodanese-like protein) has been discovered relatively recently as an integral membrane component associated to the photosynthetic apparatus of higher plants. TROL is involved in the final step of photosynthetic electron transport by binding a key energy-conversion enzyme ferredoxin:NADP⁺ oxidoreductase (FNR). This interaction enables direct transfer of photosynthetic electrons from iron-sulphur protein ferredoxin at the stromal site of photosystem I to FNR, which then hands over electrons to NADP⁺. TROL is located in two distinct chloroplast compartments – in the inner envelope of chloroplasts, in its precursor form; and in the thylakoid membranes, where it is processed completely. The determinants for its different localization have not been resolved yet, along with its role in the inner envelope. N-terminal presequence directs TROL from the site of its synthesis in cytosol to the chloroplasts. We created six N-terminal amino acid substitutions surrounding the predicted presequence processing site of TROL in order to interfere with import and to obtain a construct whose localization is limited to a single intrachloroplastic site. Wild type precursor of TROL and its e1-e6 presequence mutants were labelled with [³⁵S]-methionine during *in vitro* translation and imported into isolated intact pea chloroplasts under various conditions. Time and energy requirements for acquiring a final localization have been monitored. Also, by using different extraction methods the strength of protein to membrane association has been determined. We found that a single amino acid exchange in the presequence, Ala67 to Ile67 interferes with processing of the protein in the stroma and directs the whole pool of *in vitro* translated TROL to the single subchloroplastic compartment - the inner envelope. This result opens a possibility to engineer plants with a single protein localization site in an attempt to study the function of TROL in the chloroplast inner envelope, as well as possible consequence/s of its absence from the thylakoids.

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RSAD2 (viperin) restricts replication of a wide range of viruses via a common mechanism

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Keywords: iron-sulphur, Innate immune system, radical-SAM, viral infection, RSAD2

Science Type / Oral or Poster: Oral

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Power saturation in EPR made easy

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Keywords: EPR, power saturation, iron-sulfur proteins

EPR spectroscopy has always played a central role in the study of iron-sulfur proteins from their initial discovery in 1960 till today. An inspection of the 6-decade spanning literature indicates considerable variations in quality of the research; overall the value level does not necessarily increase over time [see: (2018) EPR of complex biological iron-sulfur systems; open access: <http://link.springer.com/article/10.1007/s00775-018-1543-y>]. The most pressing problem appears to be a frequent misunderstanding, or at least improper handling, of the phenomenon of power saturation. One of the underlying causes, and possibly the central culprit, is the manner in which power saturation is, and has been, dealt with (or ignored) in the software of commercial computerized spectrometers over the last some 25 years. I will briefly explain the physical background of power saturation, discuss its relevance for quantitative EPR spectroscopy of iron-sulfur proteins, indicate how research literature affected by improper power handling can readily be recognized, and present a simple and straightforward approach, in the form of freely available software, to practically deal with the matter with minimal effort.

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References: -

Covalent immobilization of enzymes on polymers

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Keywords: Polymer, Enzyme, covalent immobilization

The work presents results obtained on covalent immobilization of enzymes and living organisms on polymers as an example for possible cooperation inside COST 15133 for finding new applications, by covalent immobilization of iron-sulfur proteins. The covalent immobilization of Xylanase on cellulose acetate membranes was performed by grafting acrylamide on the membrane. The new ultrafiltration membranes were used for the depolymerization of Xylan to oligo-xylan, with the intention to obtain new sweeteners less dangerous for humans. Other researches with the same aim were performed by the covalent immobilization of xylanase on acrylic hydrogels and on polyvinyl alcohol fibers. Polyvinyl alcohol (PVA) is a very useful support for covalent immobilization of enzymes, because it contains many OH groups which may be easily activated with glutaraldehyde for reacting with NH_2 groups of proteins, such as enzymatic proteins. Taking into account this feature, it was performed another research on the immobilization of horseradish peroxidase on PVA fibers with the purpose to obtain antidodor textiles, the immobilized enzyme having the role to decompose unpleasant smelling compounds from perspiration. In another study, molecularly imprinted PVA membranes were deposited on central electrode of an electrochemical screen printed sensor in order to detect the use of prohibited pesticides such as atrazine. The enzyme used for this application was a phenol oxydase (tyrosinase). The great disadvantage of using PVA membrane for biosensors is that it is a dielectric polymer, so that, the transfer of the electron resulted by the redox enzymatic reaction to the electrode is difficult. In order to overpass this drawback another research was performed about the covalent immobilization of enzyme on an electroconductive polymer, such as polypyrrole. For this, polypyrrole grains, or polypyrrole membrane obtained by electropolymerization were used. Working with nitrate and nitrite reductases amperometric biosensors for nitrate and nitrite detection in water for human consumption were obtained. One of the main threat to the environment is the presence of polyphenols in waste waters, as a result of the detergents decomposition. Such waste waters are coming, for instance, from the hotels, so that, in order to reduce this danger, a study was performed about the covalent immobilization of Tyrosinase on an ultrafiltration bicomponent (acrylic-PVA) membrane.

Today, our group is performing researches for the covalent immobilization of bacteria on polymer magnetic pearls, for dyes decomposition from waste waters coming from textile industry, with the aim to collect these pearls magnetically, to regenerate them and to reuse in water treatment, avoiding at the same time the loss of the bacteria in the environment.

Science Type / Oral or Poster: Oral

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Expression, purification and mass spectrometric characterization of *Saccharomyces cerevisiae* Yah1 protein

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Keywords: protein expression, protein purification, Yah1 protein, mass spectrometry, ESI-MS

Ferredoxins are a large family of iron-sulphur cluster proteins involved in various cellular redox processes [1]. Yah1 protein (yeast adrenodoxin homolog 1) is a yeast ferredoxin that contains [2Fe-2S] iron-sulphur cluster binding domain and is the functional ortholog of bacterial ferredoxins and human Fdx2 [2]. Yah1p is involved in haem *a* biosynthesis and it is also required for the iron-sulphur cluster assembly (for both cytosolic and mitochondrial Fe-S proteins) [3]. In the current project YAH1 of *Saccharomyces cerevisiae* was cloned into HisUbi-tag containing pET24d vector and expressed in *E.coli* BL21 Star (DE3) cells. The protein purification was executed using various chromatography techniques and tag was removed with mouse ubiquitin hydrolase 2 (Usp2). Holo-protein was further characterized using MALDI-MS, however due to acidic sample preparation conditions, MALDI-MS proved to be not suitable for studying the [2Fe-2S] cluster. The loss of Fe-S cluster in acidic conditions was confirmed by UV/Vis spectroscopy. Natively purified and reconstituted holo-Yah1 was also studied with ESI-MS and various ESI-MS conditions were tested, however, Fe-S cluster was not detected, instead the major peak referring to Yah1 with 2 iron ions was observed. As the presence of the sulphide was confirmed by sulphide determination and UV/Vis spectroscopy, it was concluded that the absence of [2Fe-2S] cluster peak in ESI-MS spectra is caused due to loss of sulphide ions during the ESI-MS analysis. In conclusion, for further Fe-S cluster stability experiments with ESI-MS, the peak of Yah1 protein with two iron ions can be used, as it was proven that the cluster stability depends mainly on the binding affinity of iron ions.

Science Type / Oral or Poster: Poster

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Crystallization and preliminary structural analysis of lysine 2,3-aminomutase from *Bacillus cereus*

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Keywords: lysine-aminomutase; Fe-S cluster; biotechnology

2,3-lysine aminomutases (LAM) are iron-sulfur cluster-containing enzymes that catalyze the interconversion of L- α -lysine and β -lysine. Despite aminomutases have been suggested as potential biocatalysts, there is scarce information on the biotechnological application of LAM [1]. We have over-expressed and purified a putative LAM from *Bacillus cereus*; initial UV-spectra analysis showed the presence of PLP and a Fe-S cluster. The enzyme was crystallized and its structure solved at 2.15 Å resolution. No electronic density was found neither for the Fe-S cluster, AdoMet nor for some of the residues in this pocket; our results suggest that the cluster have been destroyed during protein storage, crystallization or data collection. However, PLP appears as its internal aldimine form bound to Lys346, confirming the previously suggested role of this residue in *Bacillus subtilis* LAM [3]. Furthermore, comparison with the only other 2,3-lysine aminomutase structure available in the PDB [2] shows interesting conformational changes for residues involved in AdoMet binding, pointing to a high plasticity of the cofactor binding pocket.

Science Type / Oral or Poster: Poster

References: [1] Hung and Lai. *J Microbiol Immunol Infect.* (2013) 46(1):1-10. [2] Lepore et al., *Proc Natl Acad Sci U S A.* (2005) 102(39):13819-24. [3] Chen and Frey. *Biochemistry.* (2001) 40(2):596-602.

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Covalent immobilization of bacterial strains upon polymer pearls bearing magnetic nanoparticles

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Keywords: polymer magnetic pearls, functionalization, bacterial strains, immobilization

Industrial and urban development yields large amounts of pollutants in the environment with harmful effects on water and implicitly affects on human health. Dyestuffs, especially azo-dyes, are some of the most important industrial pollutants, as they are hardly biodegradable, toxic and carcinogenic. In addition, the textile industry consumes large amounts of water and produces high amounts of effluents containing: suspensions of solid materials; detergents; amine; aldehydes; heavy metals and dyes. Therefore, it becomes necessary to find new, non-polluting and economical methods of water amendment, an increased interest being paid to adsorption, coagulation, photocatalytic oxidation and biodegradation [1, 2]. Biological treatment can remove significant amounts of biodegradable organic compounds from wastewater, with much lower costs than physical and chemical methods. In this work, advanced treatment means are developed using immobilized bacterial strains. The use of polymers as support materials for the immobilization of biologic components provides certain advantages: biocompatible polymers can be selected, functional groups can be easily transformed either by copolymerization or by activation with a suitable chemical agent or by blending with another polymer. In this respect, magnetic pearls were developed using a ternary mixture consisting of: AN-AMA (acrylonitrile- methacrylic acid) copolymer, PVA (polyvinyl alcohol) and Fe_3O_4 nanoparticles. Prior to immobilization pearls are undergone an activation step, by reaction with GA (glutardialdehyde; under acid catalysis) to render the required functional groups. *Pseudomonas* bacterial strains were used as biocomponent. The occurrence of immobilization and functionalization was confirmed by modern characterization techniques (FTIR, TGA, SEM).

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Science Type / Oral or Poster: Poster

References: References 1. J. L. Sims et. a al., Bioengineering for Water Clean: State-of-the-Art Assessment, Utah State University, Logan, November 1992. 2. H. Moawad et. Al., Microbial biodegradation of Reactive Blue (RB) textile azodye in sequential anoxic/aerobic bioreactor, International Journal of Advanced Research, 1 (2013) 272-284. Acknowledgment: This work has been funded by the Core project No. 18.22.03.01 Covalent immobilization of microorganisms on magnetic pearls, for remediation of wastewaters bearing dyes and by COST Action CA15133, supported by COST (European Cooperation in Science and Technology).

Characterization of mitochondrial import-export and cytosolic iron sulfur protein machineries in *Trypanosoma brucei*

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Keywords: trypanosoma, Fe-S cluster

Science Type / Oral or Poster: Oral

The monothiol glutaredoxin GrxD is involved in iron homeostasis maintenance in *Aspergillus fumigatus*

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Keywords: fungi, *Aspergillus*, iron, regulation, glutaredoxin

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). Previously, GrxD orthologs were shown to mediate cellular transport of iron sulfur-clusters and iron sensing in *S. cerevisiae* and *S. pombe*. Gene deletion attempts indicated that *grxD* is an essential gene in *A. fumigatus*, which contrasts *S. cerevisiae* and *S. pombe*. Several lines of evidence indicate that GrxD is involved in maintaining iron homeostasis in *A. fumigatus*. Expression of *grxD* was found to be upregulated during iron starvation compared to iron sufficiency. Downregulation of *grxD* expression resulted in derepression of genes involved in iron-dependent pathways and repression of genes involved in iron acquisition during iron starvation conditions, but did not significantly affect these genes during iron sufficiency. These GrxD-affected "iron"-genes are regulated by the transcription factors SreA (which represses iron acquisition during iron sufficiency) and HapX (which represses iron-dependent pathways and activates iron acquisition during iron starvation as well as activates iron-dependent pathways during iron excess). Consequently, these data indicate that GrxD is involved in iron-sensing by SreA and/or HapX. In agreement, inactivation of SreA suppressed the lethal effect of GrxD-deficiency suggesting that GrxD is essential for inactivation of SreA during iron starvation, in other words sensing of iron starvation.

Science Type / Oral or Poster: Oral

Iron sensing is governed by mitochondrial, but not by cytosolic iron-sulfur cluster biogenesis in *Aspergillus fumigatus*

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Keywords: Fungi, *Aspergillus*, Iron, Regulation

For optimal growth, microorganisms have to adapt their iron metabolism to the requirements of their ecological niche to avoid iron shortage as well as iron toxicity. Therefore, mechanisms have been evolved to tightly regulate iron uptake, consumption and detoxification, respectively, which depend on sensing the cellular iron status. In the facultative anaerobic yeast *Saccharomyces cerevisiae*, iron sensing has been shown to depend on mitochondrial (ISC) but not cytosolic iron-sulfur cluster assembly (CIA), while in mammals the cellular iron state is sensed via cytosolically synthesized iron-sulfur clusters. To address the question how the obligatory aerobic mold *Aspergillus fumigatus* senses the cellular iron state, mutant strains allowing down-regulation of ISC and CIA were generated. These studies revealed that: (i) Af-Nfs1 (Afu3g14240) and Af-Nbp35 (Afu2g15960), which are required for ISC and CIA, respectively, are essential for growth; (ii) inactivation of the Frataxin homolog Af-FxnA (Afu4g10510), which is involved in ISC, is not lethal, but results in a severe growth defect; (iii) a decrease in ISC (Af-Nfs1 depletion, Af-FxnA-deficiency) but not CIA (Af-Nbp35 depletion) results in an iron starvation response accompanied by increased iron toxicity; and, likewise, (iv) a decrease in mitochondrial iron import results in an iron starvation response. Taken together, these data underline that iron sensing in *A. fumigatus* depends on the mitochondrial, but not the cytosolic iron-sulfur cluster machinery. Moreover, depletion of the glutathione pool caused an iron starvation response underlining a crucial role of glutathione in iron sensing in *A. fumigatus*.

Science Type / Oral or Poster: Oral

Biliverdin-copper complex at physiological pH

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Keywords: biliverdin, copper, redox, coordinate, EPR

Biliverdin (BV), a product of heme catabolism, is known to interact with transition metals, but the details of such interactions under physiological conditions are scarce. Herein, we examined coordinate/redox interactions of BV with Cu^{2+} in phosphate buffer at pH 7.4, using spectrophotometry, MS-ESI, Raman spectroscopy, ^1H NMR, EPR, and electrochemical methods. BV appears to form two types of complexes with copper, both in 1:1 stoichiometry. The predominant form developed via redox reaction between BV and Cu^{2+} resulting in the formation of a coordination complex composed of a BV radical anion and Cu^{3+} . The complex showed very strong paramagnetic effects, implicating the presence of a highly delocalized e^- and a hybrid resonant structure. The structure of BV was more rigid in the complex. The complex was stable and underwent oxidation by molecular oxygen only in the presence of an excess of Cu^{2+} . The other type of the complex most likely contains Cu^{2+} coordinatively bound in the center of porphyrin ring and does not encompass an organic radical. The biological effects of a stable complex containing a delocalized electron and Cu in 3+ form should be further examined, and may provide an answer to the long-standing question of high energy investment (two electron reduction) in the catabolism of BV, which represents a relatively harmless molecule *per se*.

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Biophysical characterization of three Fe-S containing DNA repair enzymes from bacterium *Deinococcus radiodurans*

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Keywords: *D. radiodurans*, DNA repair, Base Excision Repair, Endonuclease III, Fe–S cluster

Deinococcus radiodurans is an extremely radiation and desiccation resistant bacterium, which can withstand 200 times higher doses of ionizing irradiation than other bacteria without losing viability [1]. The resistance mechanism is not known, but an efficient DNA repair machinery, which includes an elevated number of DNA glycosylases, is considered to play a key role in it. The Base excision repair (BER) mechanism is the main pathway responsible for detection and repair of oxidation damages in DNA and it is highly conserved from bacteria to man. It is initiated by Endonuclease III (EndoIII), which is ubiquitous bifunctional DNA glycosylase that belongs to the helix-hairpin-helix family of DNA glycosylases and possesses a [4Fe-4S] cluster. It has specificity for a broad range of oxidized pyrimidine lesions, removing numerous forms of damaged bases from DNA [1]. These enzymes represent an important part of the genome maintenance system in prokaryotes and eukaryotes, however, their mechanistic properties and in particular, the role of the Fe–S cluster, are not fully understood. *D. radiodurans* possesses three EndoIII like genes in its genome (EndoIII1, 2 and 3). Our recent electrochemical studies of EndoIII2 indicated that redox activation of its [4Fe-4S] cluster is DNA independent in this enzyme [2,3]. We have furthermore employed a toolbox of biophysical methodologies, including vibrational spectroscopy, electrochemistry and X-ray analysis in order to characterize all three enzymes and understand the structural basis for their functional differences. Here we present a comparative study of these three endonucleases III from *D. radiodurans*, which will ultimately allow us to disentangle their respective specific roles in this unusual phenotype.

Science Type / Oral or Poster: Oral

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Towards a full understanding of iron sulfur cluster biogenesis

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Keywords: biogenesis, frataxin, iron sulfur clusters, molecular machines, structure

Iron sulfur cluster formation is an essential and yet poorly understood molecular machine common to all living organisms and involved in several different pathways. They are formed by a complex network of weak interactions which involve evolutionary conserved proteins which in bacteria are grouped in operons. Frataxin, an essential and highly conserved mitochondrial protein whose reduced expression causes Friedreich's ataxia (FRDA) in humans, is an active part of this assembly: using a bacterial model and different biochemical and molecular biology techniques, we have proven that frataxin acts as an iron concentration dependent inhibitor of cluster formation. This suggests that frataxin is an iron sensor which acts as the gate keeper for Fe-S cluster formation and fine tunes the quantity of Fe-S clusters to the concentration and/or possibly the distribution of the available acceptors. I will review our work and the more recent advancements of our research. Our observations provide a new perspective for understanding iron sulfur cluster biogenesis and a mechanistic model which rationalizes the available knowledge.

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Glycosylation profile of human Transferrin in various pathologies and age groups

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Keywords: Transferrin, lectin-microarray, diabetes, ageing, carcinoma

Transferrin (Tf) is one of non-hem metal binding glycoproteins. This is a monomeric protein which consists of 679 amino acids and has molecular mass of approximately 79kDa. Tf is iron chelator, capable of binding two Fe^{3+} ions reversibly. It has two N-glycosylation sites at Asn⁴¹³ and Asn⁶¹¹, normally occupied by biantennary complex-type structures terminating with α 2,6-linked N-acetylneuraminic acid (NeuAc). Tf isolated from particular patients is already regarded as a biomarker for determination of congenital disorders of glycosylation (CDG), alcohol abuse (prominent carbohydrate-deficient transferrin, CDT) and also a perspective molecule in investigation of other pathologies (diabetes, some types of carcinoma) and ageing. Also, perturbations in Tf glycosylation could potentially lead to changes in its affinity towards Fe^{3+} ions.

Isolation of Tf from serum samples was done by three step precipitation procedure developed in our laboratory. Lectin-microarray analysis of isolated Tf from patients with diabetes, colon carcinoma and both male and female participants of different age revealed statistically relevant changes in glycosylation of this molecule. We successfully established lectin-microarray protocol, where most relevant lectin-glycan interaction were identified (six out of eleven used lectins) and also, appropriate Tf concentration necessary for precise and accurate analysis.

Our analysis showed significant changes in fucosylation of Tf in diabetes and colon carcinoma patients compared to the control sample. Also, fucosylation appears to be gender independent but with significant differences between age groups. Significant changes were also detected in the case of sialylation (diabetics vs control, and in ageing) and in the content of galactose, N-acetylglucosamine and mannose (diabetics vs control, colon carcinoma patients vs control and in ageing).

Having in mind that changes in protein glycosylation cause alterations in its function, lectin microarray analysis could point out to Tf molecules as suitable candidates for further functional analysis on both circulation and cellular level.

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