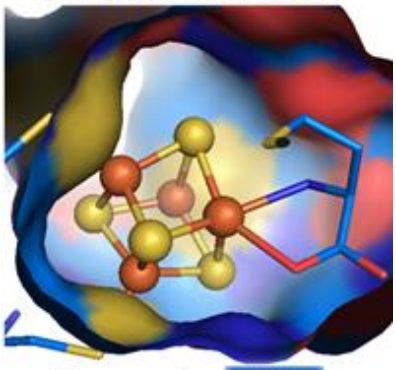
FeSImmChemNet



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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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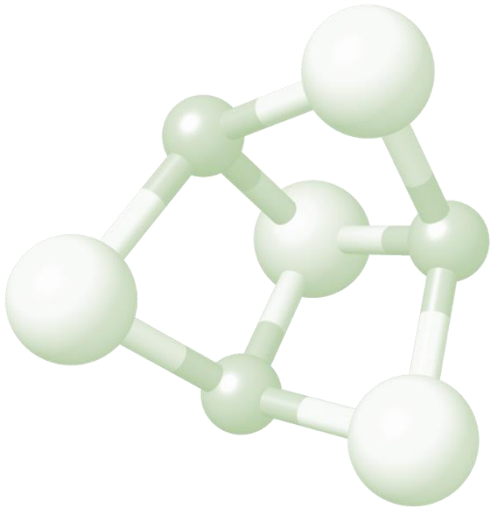
1st Meeting

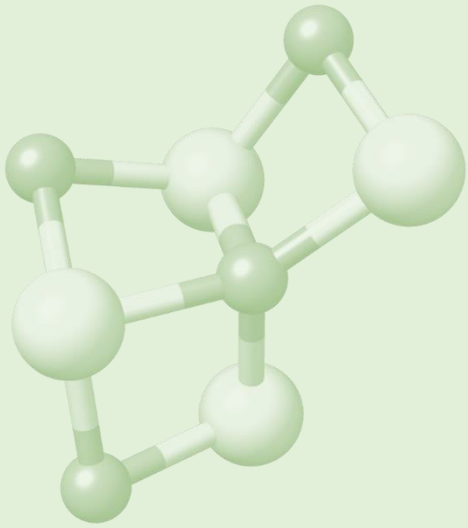
FeS Clusters from Chemistry to Biology and Beyond



Program & Book of Abstracts

23-25 February 2023

Hotel Riviera, Carcavelos, Portugal



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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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Welcome

It is with great pleasure to welcome each of you to the first meeting of the COST Action Iron-sulfur Clusters, From Chemistry to Immunology (FeSImmChemNet). As we celebrate the start of our Action, this meeting is a landmark step towards achieving the Action's objectives. We aim to develop and coordinate a multidisciplinary pan-European Network consisting of research groups from different disciplines, including stem-cell technology, immunology and virology, metabolomics, bioinorganic chemistry, computational chemistry, structural biology, and medicinal chemists. We can only address global challenges in human diseases and build a sustainable and prosperous Europe through such a coordinated effort.

In the next few days, you will hear from leading groups across Europe about their research and discoveries describing:

(i) The role of metals and iron-sulfur clusters in the immune system and infectious diseases;
(ii) Various methods and techniques used to study the functions of metals and iron-sulfur clusters and proteins in biology;
(iii) Iron metabolism and biosynthesis of iron-sulfur clusters
(iv) Iron-sulfur proteins and clusters as potential targets to treat human diseases like viral infection and cancer
(v) Technologies and methods used for drug delivery and targeting iron-sulfur proteins

Let me give you a brief update on our progress since the start of Action in November 2022. We are proud that the Action has more than 120 members and includes 22 countries, of which 14 are from COST Inclusive Target Countries, Near Neighbour Countries, and Partner Members. We are happy to announce that the Action grants are now open for application. We will support Short-Term Scientific Mission Awards, Visual Mobility Grants, ITC Conference Grants, and Dissemination Conference grants. Finally, the action website is now published (www.fesimmchemnet-cost.com), where you can find more details about the Action and future activities.

Finally, I thank you for attending this meeting and sharing your research and expertise. Addressing challenges and achieving the objectives of the Action is only possible with your collective knowledge and expertise to cross boundaries of bioinorganic chemistry and immunology/virology. With your support and leadership, we will be able to strengthen European's research and innovation capacities with an emphasis on overcoming bottlenecks in geographical, age, and gender.

I hope that we will all enjoy the meeting in Carcavelos and that it will pave the way for many collaborations and scientific achievements. I look forward to meeting each of you.

Sincerely Yours,

Dr Kourosh H. Ebrahimi

Action Chair

Action FeSImmChemNet

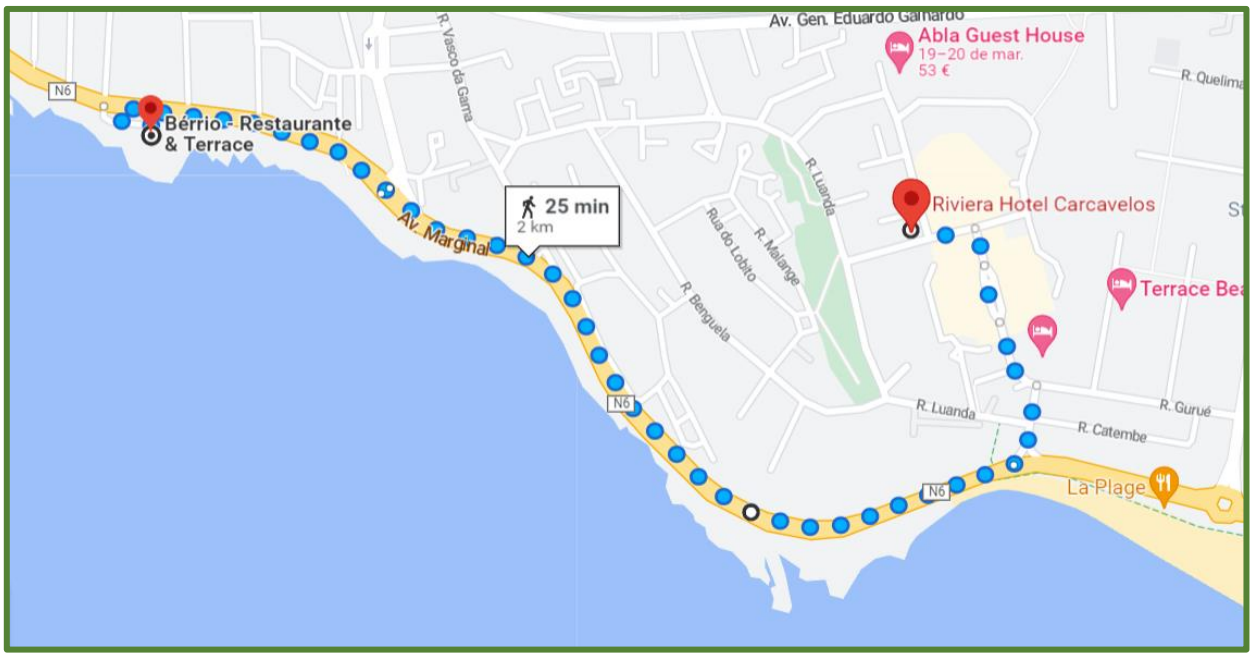
Venue / General Information

Registration Desk, in front of *Safira* room (1st floor), will remain open throughout the meeting.

We ask speakers to bring their slides to the *Safira* room from 8:30 to 9:00 am for morning sessions and from 2:00 to 2:30 pm for the afternoon sessions.

Posters should be fixed on the 23rd Feb during lunch time (12:30 to 2:30 p.m.) in *Esmeralda* room. Poster session will take place on the 23rd Feb from 5:35 to 7:35 p.m.

Joint CA dinner will take place on the 24th Feb at 7:30 p.m. and will be served at **Bérrio - Restaurante & Terrace**, Avenida Marginal - Praia da Parede.



Program

23 February 2023

08:30 – 9:00 Registration

Chair: Fred Hagen

09:00 – 9:25 Kourosh Ebrahini | King's College London, UK
CA Chair welcome note

09:30 – 9:50 Sally Cowley | Sir William Dunn School of Pathology, University of Oxford, UK
Human iPS cells as a tool for understanding FeS cluster protein function in innate immunity

09:55 – 10:15 Sandrine Ollagnier | Univ Grenoble Alpes, FR
Metabolism of Fe-S clusters in Mycobacterium tuberculosis: the SUF machinery as a good therapeutic target

10:20 – 10:40 Mario Piccioli | Magnetic Resonance Center, University of Florence, IT
Relaxation-based NMR assignment: spotlights on ligand binding sites in human CISD3 and cluster binding residues in PioC

10:45 – 11:15 Coffee Break

Chair: Elin Moe

11:20 – 11:40 Megan Teh | MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK
Iron restricted CD8+ T-cells display metabolic perturbations and show sensitivity to aspartate supplementation

11:45 – 12:05 Benoit D'Autréaux | Institute for Integrative Biology of the Cell, Université Paris-Saclay, FR
Functional role of a Fe-S cluster in a rotavirus non-structural protein

12:10 – 12:30 Ralf Erik Wellinger | Centro Andaluz de Biología Molecular y Medicina Regenerativa, ES
Iron-sulfur clusters and genome stability

12:30 – 2:30 Lunch (Poster Fixing)

Chair: Simone Ciofi

2:30 – 2:50 Peter-Leon Hagedoorn | Delft University of Technology, NL
Advanced methods to explore viral metalloproteins

2:55 – 3:15 Kleva Shpati | Albanian University, AL
Phytochemical compound from medicinal plants can reached health benefits

3:20 – 3:40 Sofia Pauleta | NOVA School of Science and Technology, Universidade NOVA de Lisboa, PT
Fe/S proteins from strict anaerobic bacteria – the ORP molecular system

3:45 – 4:05 Holger Stark | Heinrich-Heine-Universität Düsseldorf, DE
Multiple targeting in neurodegenerative diseases

4:10 – 4:40 Cofee Break

Chair: Goran Miljus

4:45 – 5:05 Maxie Roessler | Imperial College London, UK
Putting spins on FeS clusters to understand their properties and structure

5:10 – 5:25 Oliver Štrbák | Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, SK
Inflammation, iron-sulfur cluster deficits, and iron accumulation as envoys of neurodegeneration

5:35 – 7:35 Poster Session (Coffee Break)

24 February 2023

Chair: Ricardo Louro

09:15 – 9:45 Invited talk: Petra Wendler | Institute of Biochemistry, University of Potsdam, DE
Metalloprotein structure determination using cryo-EM: hidden gems

09:50 – 10:10 Nick Le Brun | School of Chemistry, University of East Anglia, UK
The mechanism of iron-sensing via the [4Fe-4S] cluster of the Rhizobial global iron regulator RirA

10:15 – 10:35 Iztok Turel | Faculty of Chemistry and Chemical Technology, University of Ljubljana, SL
Metal-pyrithione complexes, Fe-S clusters and SARS CoV-2

10:40 – 11:10 Coffee Break

Chair: Antonio Pierik

11:15 – 11:35 Yvain Nicolet | Institut de Biologie Structurale, Univ Grenoble Alpes, FR
Structure-function relationships of radical SAM enzymes

11:40 – 12:00 Suzanne Cloonan | Trinity College Dublin, IE
Iron and immunometabolism in the lung

12:05 – 12:25 Giorgio Caserta | Technical University Berlin, DE
Importance of Fe-S clusters for the catalytic function of [NiFe]-hydrogenases and their possible role in pathogenesis

12:30 – 2:30 Lunch

Chair: Beatrice Py

2:30 – 2:50 Smilja Todorovic | ITQB NOVA, Universidade NOVA de Lisboa, PT
Resonance Raman spectroscopy of Fe-S cluster containing proteins, from unknown to unusual

2:55 – 3:15 Anna Schurich | King's College London, UK
Metabolic regulation of human T cell function

3:20 – 3:40 Ingrid Span | Friedrich-Alexander-Universität Erlangen-Nürnberg, DE
Maturation strategy influences expression levels and cofactor occupancy in Fe-S proteins

3:45 – 4:05 Maria Andrea Mroginski | Technical University Berlin, DE
Computational studies of FeS cluster containing protein DCCP

4:10 – 4:40 Coffee Break

Chair: Francesca Camponeschi

4:45 – 5:05 Oliver Stehling | Institut für Zytobiologie, Philipps-Universität, DE
Functional analyses of viperin and lipoyl synthase highlight cellular requirements for the activity of radical SAM enzymes

5:10 – 6:15 Kourosh Ebrahimi | King's College London, UK
CA Chair concluding remarks

WG1-5 discussions

7:30 – CA Dinner

25th February 2023

9:30 – 10:30 Core Group meeting

10:30 – 11:00 Coffee Break

11:00 – 12:30 MC meeting

Book of Abstracts

METALLOPROTEIN STRUCTURE DETERMINATION USING CRYO-EM: HIDDEN GEMS

Petra Wendler

University of Potsdam
Department of Biochemistry, Institute of Biochemistry
Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

petra.wendler@uni-potsdam.de

Cryo-electron microscopy (EM) has greatly progressed the field of structural biological in recent years. Structures of model protein complexes can now be solved to atomic resolution, and many metalloprotein complex structures have been determined to 2-4 Å resolution using this technique. The cryo-EM structure of the soluble *Rhodobacter capsulatus* formate dehydrogenase (*Rc*FDH) at 3.3 Å resolution reveals a 360 kDa dimer of FdsABGD heterotetramers with a putative interconnection of electron pathway chains [1]. Electrons gained from oxidation of formate to carbon dioxide at the active site molybdenum atom are transferred via numerous FeS clusters and a flavin mononucleotide (FMN) onto nicotinamide adenine dinucleotide (NAD^+). The reaction is reversible and reduction with NADH results in formation of paramagnetic MoV species, as well as partial reduction of the Fe-S clusters and FMN. Due to the sensitivity of electron scattering to the coulomb potential of an atom, cryo-EM can distinguish charged states of atoms [2,3]. Comparison of the EM map of air oxidized FDH inhibited by sodium azide with that of an enzyme additionally treated with NADH shows qualitatively the charge differences at the electron accepting atoms in the electron transfer chain between the two maps. Further development of the method should allow a quantitative assessment of the relative distribution of electrons between the different iron-sulphur clusters of metalloprotein complexes and be able to resolve the charge changes caused by the introduced electrons at the atomic level.

[1] Radon C., Mittelstädt G., Duffus BR, Bürger J, Hartmann T., Mielke T, Teutloff C, Leimkühler S, Wendler P (2020) *Nat. Commun*. 11:1912.
[2] Yonekura, K., & Maki-Yonekura, S. (2016). *Journal of Applied Crystallography*, *49*(5), 1517–1523.
[3] Wang, J. (2017). *Protein Sci*, *26*(6), 1098–1104

HUMAN IPS CELLS AS A TOOL FOR UNDERSTANDING FES CLUSTER PROTEIN FUNCTION IN INNATE IMMUNITY

Sally A. Cowley

James and Lillian Martin Centre for Stem Cell Research
Sir William Dunn School of Pathology
University of Oxford, UK

sally.cowley@path.ox.ac.uk

Human Pluripotent Stem cell (iPSC) technology, combined with CRISPR-Cas9, provides a hugely exciting opportunity to interrogate human cellular physiology and pathology in vitro in authentic somatic cell lineages. Harnessing this potential, my team at Oxford specialise in using iPSC for modelling disease, particularly innate immunity, host-pathogen interactions, and neuroinflammation (important in neurodegenerative diseases). We have pioneered protocols for the efficient differentiation of macrophages from human induced Pluripotent Stem Cells, and have adapted these protocols for modelling the brain-resident cousins of macrophages, microglia, in monoculture and also in coculture with iPS-neurons. Microglia are increasingly implicated in neurodegenerative disease, particularly Alzheimer's, with numerous disease-associated genes being expressed in microglia/macrophage lineages. iPS-cell macrophages and microglia can be produced at scale, and as terminally differentiated, karyotypically normal cells with an authentic phenotype that faithfully recapitulates the genetic background of the donor, are well suited for examining the function of gene products in innate immunity and the diverse roles of disease-associated genes. FeS cluster-proteins are associated with anti-viral responses in these cells (e,g, RSAD2, aka SAND) and with mitochondrial function/regulation of mitophagy (e.g MitoNEET), i.e. are relevant to neurodegenerative disease mechanisms, hence our lab's interests relate to WG1 'FeS clusters in the immune system'. We are willing to train COST members on protocols for using iPSC and CRISPR-Cas9 to generate relevant Knockouts and mutations for study in these authentic cell models.

IRON-SULFUR METABOLISM IN MYCOBACTERIUM TUBERCULOSIS: CHARACTERIZATION OF SUFU AND SUFS PROTEINS

Sandrine Ollagnier de Choudens

Univ. Grenoble Alpes, CNRS, CEA,
Laboratoire de Chimie et Biologie des Métaux,
F-38000 Grenoble, France

sollagnier@cea.fr

Iron-sulfur (Fe-S) clusters are inorganic cofactors that are essential in various biological processes (e.g., DNA repair, respiration, photosynthesis, cofactor biosynthesis) [1]. They exist in different forms and oxidation states, the most common types being [4Fe-4S] and [2Fe-2S] clusters. In bacteria, two major pathways for Fe-S cluster biogenesis and delivery have been identified: the ISC and the SUF systems. In Escherichia coli, the ISC system is the housekeeping pathway for Fe-S biogenesis, whereas the SUF system is active under environmental stress and iron limitation [2]. Interestingly, Mycobacterium tuberculosis (Mtb), the causative agent of Tuberculosis (TB), contains only the SUF system as Fe-S assembly machinery [3]. Mtb suf operon is composed by seven genes, namely Rv1460(sufR), Rv1461(sufB), Rv1462(sufD), Rv1463(sufC), Rv1464(sufS), Rv1465(sufU) and Rv1466(sufT). This system is essential for in vitro growth of Mtb under normal conditions [4,5] and recently it proved to be a point of vulnerability [6]. Moreover, the Mtb SUF components are induced during iron starvation [7] and up-regulated under nitrosative and oxidative conditions [8], stresses encountered by the pathogen in the host. Therefore, there is mounting evidence that the SUF system is essential for Mtb pathogenicity and targeting it might open novel avenues for the development of novel anti-TB drugs through disturbing the pathogen's Fe-S metabolism. Few SUF proteins have been characterized so far. SufR was reported to be the regulator of the suf operon [9] and SufT characterized as a potential chaperone [10]. Among the Mtb SufS, SufU, SufB, SufC and SufD proteins that remain to be characterized, recent results on SufS and SufU proteins will be presented.

[1] Johnson DC., et al. (2005) Annu. Rev. Biochem. 74: p. 247-281;
[2] Py, B. and F. Barras. (2010) Nat. Rev. Microbiol.. 8(6): 436-446;
[3] Huet G., et al. (2005), J. Bact., 7(17): 6137-6146;
[4] Sassetti CM., et al. (2003), Mol. Mic., , 48, 77-84 ;
[5] Griffin JE., et al. (2011) PLoS Pathog.; 7:e1002251;
[6] Bosch B., et al. (2021) Cell, , 184, 4579-4592 ;
[7] Kurthkoti, K., et al. (2017) mBio, 8(4): p. e01092-17 ;
[8] Cortes, T., et al. (2017) Sci Rep,. 7: 8208 ;
[9] Willemse D., et al. (2018), PLoS One, 6;13(7):e0200145 ;
[10] Tamuhla, T. et al. (2020), Microbiology, 166, 296-305.

RELAXATION-BASED NMR ASSIGNMENT: SPOTLIGHTS ON LIGAND BINDING SITES IN HUMAN CISD3 AND CLUSTER BINDING RESIDUES IN PIOC

Grifagni D. [1,2], Trindade, I.B. [3], Querci, L. [1], Silva J.M. [1,2], Louro, R.O. [3], Cantini F. [1,2], Piccioli M.[1,2]

[1] *Department of Chemistry (DICUS) University of Florence, Sesto Fiorentino, Italy*
[2] *Magnetic Resonance Center, University of Florence, Sesto Fiorentino, Italy*
[3] *Instituto de Tecnologia Química e Biológica* António Xavier *da Universidade Nova de Lisboa, Avenida da República (EAN), 2780-157 Oeiras, Portugal*

piccioli@cerm.unifi.it

NMR is a powerful technique to obtain information at the atomic level in solution on the residues involved in intermolecular interactions and on the fine structural details/rearrangements. In FeS proteins, this is limited by signal broadening around the paramagnetic centers. Tailored experiments revive signals of the cluster surrounding; however, signals identification without specific residue assignment and without the possibility to convert the NMR information into structural restraints remains useless.
CISD3 is a mitochondrial protein belonging to the NEET proteins family, bearing two [Fe_2S_2] clusters coordinated by CDGSH domains. At variance with the other proteins of the NEET family, very little is known about its structure-function relationships. Here, paramagnetic relaxation drives the signal assignment of residues in the proximity of the paramagnetic center(s) and allowed us to identify the potential key players of the biological function of the CISD3 protein.
It is well known that relaxation rates can be converted into electron spin-nuclear spin distance restraints, also known as Paramagnetic Relaxation Enhancement (PRE) restraints. The High Potential Iron Sulfur Protein (HiPIP) PioC is a case study to discuss the complementarity of ^{13}C PRE restraints with ^{1}H PRE restraints. ^{13}C R_1 values can be measured also at very short distances from the paramagnetic center and that the obtained set of ^{13}C based restraints can be added to ^{1}H PREs and to other classical and paramagnetism based NMR restraints to improve quality and quantity of the NMR information.

IRON RESTRICTED CD8+ T-CELLS DISPLAY METABOLIC PERTURBATIONS AND SHOW SENSITIVITY TO ASPARTATE SUPPLEMENTATION

Megan Teh[1], Joe Frost[1], Linda Sinclair[2], Nancy Gudgeon[3], Barbara Kronsteiner-Dobramysl[4], Jennie Roberts[3], Jan Rehwinkel[1], Susanna Dunachie[4], Sarah Dimeloe[3], Andrew Armitage[1], Hal Draksmith[1]

[1] MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK; [2] Cell Signalling and Immunology, University of Dundee, Dundee, UK; [3] Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK; [4] Centre for Tropical Medicine and Global Health, Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, UK

megan.teh@ccc.ox.ac.uk

Iron-deficiency affects ~2 billion people [1] and ~2% of human genes encode iron-interacting proteins, 17% of which bind iron-sulfur (Fe-S) clusters. Fe-S cluster binding proteins are involved in processes including mitochondrial metabolism, DNA synthesis and repair and the cellular response to stress [2]. Low iron profoundly impairs T-cell immunity [3], but the mechanisms underlying this phenotype remain unclear. We conducted transcriptomic and proteomic screens of iron-deficient murine CD8+ T-cells to identify aspects of biochemistry most impacted by iron restriction. T-cells cultured in iron depleted conditions showed suppression of genes involved in mTORC1 and MYC signalling, suggesting that iron scarcity may modify T-cell metabolism. Proteomic analysis also revealed upregulation of mitochondrial proteins involved in oxidative-phosphorylation and beta-oxidation, many of which contain Fe-S clusters. Combined with published data indicating that iron-restriction suppresses mitochondrial but not glycolytic ATP synthesis, we proposed that mitochondrial function may be particularly sensitive to iron-deficiency. We found that iron restricted T-cells have elevated mitochondrial reactive oxygen species, suggestive of electron transport chain dysfunction. We hypothesise that this effect may be driven by elevated levels of mitochondrial proteins in the absence of the necessary Fe-S and heme cofactors. Iron starved T-cells also showed depletion of the mitochondrial tricarboxylic acid (TCA) cycle metabolites, alpha-ketoglutarate and malate, the synthesis of which lies downstream of the Fe-S-dependent enzymes, aconitase and succinate-dehydrogenase, respectively. Supplementation of aspartate, an essential TCA cycle product, substantially ameliorated the inhibitory effects of iron-deficiency on T-cell proliferation and effector function. Our data indicates that iron restriction impairs mitochondrial function at multiple nodes, but that specific interventions can overcome sensitivity to iron-deficiency. Our work provides metabolic mechanisms that partially explain the inhibition of T-cell responses by iron deprivation.

[1] Vos, T. et al., 2016, The Lancet, 390, 1211-1259.
[2] Andreini, C., Putignano, V., Rosato, A. & Banci, L., 2018, Metallomics, 10, 1223-1231
[3] Frost, J. N. et al., 2021, Med, 2, 164-179

FUNCTIONAL ROLE OF A FE-S CLUSTER IN A ROTAVIRUS NON-STRUCTURAL PROTEIN

Davy Martin, Annie Charpilienne, Aubérie Parent, Alain Boussac, Joël Poupon, Didier Poncet, Benoit D'Autréaux

Institute for Integrative Biology of the Cell (I2BC),
Université Paris-Saclay, CEA, CNRS,
91198 Gif-sur-Yvette, France

benoit.dautreaux@i2bc.paris-saclay.fr

During rotavirus infection, replication and packaging of the viral genome are performed in viral factories, termed viroplasms. The viral non-structural protein NSP5 is a major building block of viroplasms; it recruits the viral polymerase VP1, the core protein VP2, and the ATPase NSP2 inside the viroplasm to form the viral replication complex. Here we report that NSP5 is a unique viral metalloprotein that coordinates a [2Fe-2S] iron-sulfur cluster as demonstrated by the metal and labile sulfide contents, UV-visible light absorption, and electron paramagnetic resonance.[1] We have analyzed the role of the Fe-S cluster of NSP5 in RNA binding using microscale thermophoresis. We found that the iron-sulfur cluster modulates the affinity of NSP5 for single-stranded RNA. Because the cluster is near the binding sites of both the polymerase VP1 and the ATPase NSP2, we anticipate that this cluster is crucial for NSP5 functions, in either packaging or replication of the viral genome.

These data highlight that viruses are able to hijack the Fe-S cluster assembly machineries of host cells to acquire their Fe-S clusters. Thereby targeting Fe-S cluster biogenesis and the connection between these machineries and viral protein could be a novel strategy to develop anti-viral drugs. I will present a high throughput-screening assay that enables discovery of molecules specifically inhibiting the early steps of the Fe-S cluster biosynthetic process in eukaryotic cells.[2,3]

[1] Martin D., Charpilienne A., Parent A., Boussac A., D'Autreaux B., Poupon J., Poncet D. (2013), FASEB J., 27, 1074-1083
[2] Gervason S., Larkem D., Schunemann V., Cianferani S., Sizun C., D'Autreaux B. (2019), Nat Commun, 10, 3566
[3] Monfort B., Want K., Gervason S., D'Autreaux B. (2022), Front. Neurosci., 16, 838335

'IRON-SULFUR CLUSTERS AND GENOME STABILITY'

Hélène Gaillard, Inés G. de Oya and Ralf E. Wellinger

Centro Andaluz de Biología Molecular y Medicina Regenerativa,
Universidad de Sevilla,
Avda. Américo Vespucio s/n, 41092 Sevilla, Spain

ralf.wellinger@cabimer.es

Genomic instability is characterized by a range of DNA alterations and established as a hallmark of cancer and aging. These DNA alterations include point mutations, INDELs or whole chromosome numerical changes, which irreversibly change the information content of the genome. Defects at different stages of the mitochondrial Fe-S cluster assembly machinery (ISC) activate DNA damage signaling pathways suggesting the presence of constitutive DNA lesions, and lead to increased spontaneous mutation rate and hyper-recombination. Moreover, Fe-S clusters are essential cofactors of many proteins involved in DNA replication, repair and recombination. Thus, impaired Fe-S cluster synthesis and assembly appear to challenge genome maintenance by increased DNA damage and impaired DNA synthesis and repair, respectively. Budding yeast has served as an excellent model organism to study the impact of Fe-S cluster synthesis on genome instability. In this presentation, I will discuss the genetic and molecular tools that allow us to study the role of Fe-S clusters in genome stability.

ADVANCED METHODS TO EXPLORE VIRAL METALLOPROTEINS

P.-L. Hagedoorn

Department of Biotechnology,
Delft University of Technology,
Van der Maasweg 9, 2629HZ, Delft, The Netherlands

p.l.hagedoorn@tudelft.nl

In recent years evidence has emerged that metalloproteins are important in viral infection and replication and in our immune response. This important new field of science can be unlocked using bioinorganic chemical techniques that focus on the metal in the biological environment and measure the identity, quantity, electronic structure and micro-environment. Two different approaches and their potential to explore viral metalloproteins will be presented. To explore the microbial metalloproteome we developed the metalloproteomics approach MIRAGE (Metal Isotope native RadioAutography in Gel Electrophoresis). Using MIRAGE we have been able to explore the soluble Cu, Fe and Zn metalloproteome of Escherichia coli (Fig. 1) [1] and Mo and W metalloproteome of Pyrococcus furiosus [2]. MIRAGE technology has been developed to separate and quantify the native proteins associated with a particular transition metal ion from a cellular system.

Ultrafast mixing techniques were used to establish kinetic mechanisms of metalloenzyme catalysis. Two different in-house developed ultrafast kinetic techniques were used: Nanospec (deadtime ca. 3 µs), ultrafast continuous flow UV-vis spectrophotometry; MHQ (deadtime ca. 80 µs), microsecond timescale rapid freeze hyperquenching [3]. The dead times of these instruments are 100X shorter than for commercially available devices. Novel intermediate states were identified in the catalytic mechanism of the fast heme enzyme chlorite dismutase [4]. Chlorite dismutase is a unique heme b dependent enzyme that catalyzes the conversion of chlorite (ClO^{2-}) to molecular oxygen (O_2) and chloride (Cl^-). This reaction involves O-O bond formation, which is rare in nature. The enzyme catalyzes a single turnover in less than a millisecond. Unlocking the role of [Fe-S] clusters in viral infection and replication will require the experimental probing of Fe in the cell and characterization of (recombinantly expressed) viral FeS proteins. Radioactive Fe is an attractive probe due to its sensitivity and selectivity. Using MIRAGE it will be possible to quantitatively establish the Fe distribution among host cell proteins at different stages of viral infection. Ultrafast mixing techniques allow the determination of the kinetic mechanisms of metalloenzyme catalysis and metal binding to proteins.

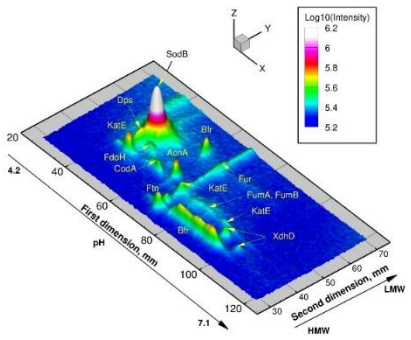


Figure 1. 3D image of 59Fe-MIRAGE of *E. coli* soluble protein extract (575 µg protein)

[1] Sevcenco, A. M.; Pinkse, M. W. H.; Wolterbeek, H. T.; Verhaert, P. D. E. M.; Hagen, W. R.; Hagedoorn, P. L., Metallomics 2011, 3, 1324-1330. doi:https://doi.org/10.1039/C1MT00154J
[2] Sevcenco, A. M.; Pinkse, M. W.; Bol, E.; Krijger, G. C.; Wolterbeek, H. T.; Verhaert, P. D. E. M.; Hagedoorn, P. L.; Hagen, W. R., Metallomics 2009, 1, 395-402.
[3] Srour, B.; Strampraad, M. J. F.; Hagen, W. R.; Hagedoorn, P.-L., J. Inorg. Biochem. 2018, 184, 42-49. doi:https://doi.org/10.1016/j.jinorgbio.2018.04.011
[4] Püschmann, J.; Mahor, D.; de Geus, D. C.; Strampraad, M. J. F.; Srour, B.; Hagen, W. R.; Todorovic, S.; Hagedoorn, P.-L., ACS Catalysis 2021, 11, 14533-14544. doi:https://doi.org/10.1021/acscatal.1c03432

PHYTOCHEMICAL COMPOUND FROM MEDICINAL PLANTS CAN REACHED HEALTH BENEFITS

Kleva Shpati,

Albanian University,
Str Kajo Karafili No. 22 Tirana, Albania

Introduction. Medicinal plants with immune bosting effects, antimicrobial, antiviral are a group of medicinal herbs used frequently in healthy individuals and patients post Covid-19 and long Covid-19, during the pandemic year 2020 in HealthCare Clinics and pharmacies of Tirana. The aim of this study was to collect data for benefits of medicinal plants for immune bosting effects, antimicrobial and antiviral effects. The medicinal plants have been used a lot in populations, despite the skepticism for using them. **Methodology:** The study was based on a survey of the prescriptions form family doctor in 5 HealthCare Clinics and recommendations of pharmacists (188 pharmacies) during the Period March-June 2020 and October-December 2020. This study exclude Covid Therapy, and include prescriptions medicinal plants as an supplement **Results** shown that the most used medicinal plants were *Salvia Officinalis* 36%, *Plantago L 24%, Laurus Nobilis 1*4%, *Vaccinium Myrtilis* 12%, *Rosa Canina 8*% the rest 6%. They have improved the anti-infective, antiviral and boosting immune system for the individual and patients participating in this study, according to data collections from healthcare clinics. **Conclusions** Many medicinal plants are still available with health profits for antimicrobial, antiviral effects and immune boosting effects. The medicinal plants with their phytochemical derivates are an open and immense space for researchers.
Key words: Medicinal plants, Antiviral, Anti-infective Effects, Immune Bosting Effects

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Fe/S Proteins from strict anaerobic bacteria – the ORP molecular system

Sofia R. Pauleta[1,2]

[1] *Microbial Stress Lab, UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal;* [2] *Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.*

sofia.pauleta@fct.unl.pt

The Orange Protein operon encodes several metalloproteins putatively involved in the cell division of sulfate reducing bacteria and senses redox state of the cell [1,2]. The Orange protein from *Desulfovibrio vulgaris* Hildenborough forms a physiologic protein complex *in vivo* with five conserved proteins from the same gene cluster [1]. Indeed, some of these proteins cannot be separated, forming the core complex composed by Orange Protein and two ATPases, containing Fe-S centers. The Orange Protein is a 12 kDa protein that can bind non-covalently mixed metal sulfur clusters [3-5]. The 32 kDa iron-sulfur proteins are high homologous and contained MinD-like domains, such as the ones observed in proteins that participate in *E. coli* cell division. In fact, the iron-sulfur clusters of the ORP complex from *D. vulgaris* Hildenborough are oxygen sensitive, and therefore the complex have been isolated and characterized under anoxic conditions. Besides this core group of proteins, the other two also contain Fe-S clusters: DVU2105 (a small protein has been characterized from the homologous *D. desulfuricans* G20 [6]), and DVU2109, the larger protein that belongs to the Mrp/NMP35 family [7] and might be involved in iron-sulfur biogenesis.
The work presented here includes the biochemical, spectroscopic, and electrochemical characterization of the core ORP complex. The results indicate the presence of 4 x [4Fe-4S] clusters that shown unusual temperature dependence EPR signals for these types of clusters. A redox potential of -400 mV ± 5 mV was determined by electrochemical methods and potentiometric titration. Moreover, the ability to hydrolyze nucleotides was tested, showing that the complex can hydrolyze ATP. From the ATP concentration dependence study, the following kinetics parameters were determined: K_M = 0.31 ± 0.12 mM and V_{max} = 221 ± 5 pmol min^{-1} mg^{-1}.

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MULTIPLE TARGETING IN NEURODEGENERATIVE DISEASES

Holger Stark

Heinrich Heine University Düsseldorf,
Institute of Pharmaceutical and Medicinal Chemistry,
Universitaetsstrasse 1, 40225 Duesseldorf, Germany

E-mail: stark@hhu.de

Neurodegenerative diseases are immense burden for the patients and globally also for the socio-economic health systems. Although great progress has been made on the aetiology of numerous diseases like Alzheimer´s disease or Parkinson´s disease, a causal treatment is still missing and of high medical need. Instead of one target, numerous targets have simultaneously to be addressed for a sufficient therapeutic approach [1].
In addition to lipid signaling [2], we are focusing on aminergic GPCRs as primary targets on which depending on the demands of the pharmacological profile additional targets such as GPCRs, enzymes or epigenetics are addressed. The combination of pharmacophores in small molecule ligands has successfully been accomplished in different compound classes. A main focus is on the combination of histamine H_3 receptor antagonist pharmacophores with additional pharmacodynamics and pharmacokinetic properties [3,4].
In a new approach, we will take structural elements from known ligands modifying FeS proteins like MitoNEET, SAND etc. in combination to aminergic GPCR ligands (with emphasis on histamine H_3 receptors). Additional redox properties of these ligands and related compounds will be helpful in optimization of the biologically active compounds.
A broad pharmacological screening will be necessary to find the optimal profile for potential drugs.

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MECHANISTIC INSIGHTS INTO FeS ENZYMES FROM EPR SPECTROSCOPY

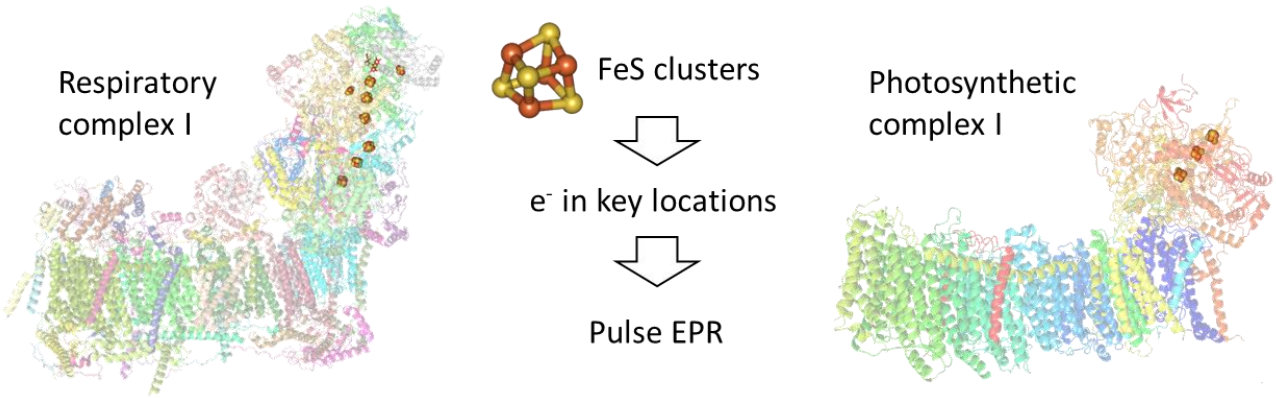
Maxie M. Roessler

Department of Chemistry,
Imperial College London,
Molecular Sciences Research Hub,
London, UK

m.roessler@imperial.ac.uk

Unpaired electrons play an important role in a wide range of redox-driven catalytic processes in FeS proteins. Controlling their location and exploiting the interactions with their environment can provide key mechanistic information into these catalytic reactions. In this talk, I will discuss how we have used and developed EPR-based techniques to gain mechanistic insights into FeS enzymes [1].

Respiratory complex I is essential for respiration in all higher organisms, but despite structural advances its mechanism, in particular how electron transfer links to the proton translocation required for ATP synthesis, is not fully understood. In conjunction with other techniques, EPR enables us to probe the roles of FeS electron transfer centres and semiquinones in this enzyme [2,3]. With the tools developed for respiratory complex I, and exploiting our advances in spin sensitivity [4], we determine the energetic profile of electron transfer in photosynthetic complex I [5] – the homologous enzyme in plants and cyanobacteria that plays a crucial role in increasing ATP production under stress.



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INFLAMMATION, IRON-SULFUR CLUSTERS DEFICIT AND IRON ACCUMULATION AS ENVOYS OF NEURODEGENERATION

Oliver Strbak[1], Jan Gombos[2], Alica Lokajova[2]

[1] Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala hora 4, 036 01 Martin, Slovakia; [2] Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala Hora 4, 036 01 Martin, Slovakia

oliver.strbak@centrum.cz

Iron accumulation, mitochondrial dysfunction, inflammation, and oxidative stress represent the common features of various neurodegenerative diseases (ND), including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), Friedrich's ataxia (FA), and neurodegeneration with brain iron accumulation (NBIA) [1]. However, it is still uncertain whether iron accumulation is the cause or consequence of previous pathology. Since the mitochondria are the major iron recipient of the cell, mitochondrial dysfunction, characterized by abnormal iron status, is associated with above mentioned neurodegenerative disorders [2]. Mitochondrial dysfunction results in a decreased synthesis of ATP, iron-sulfur clusters (ISC), and heme prosthetic groups [3]. However, except for FA [4] and NBIA [5], the correlation between decreased ISC synthesis on the one side and increased iron accumulation on the other has not been generally confirmed in ND. Since inflammation has been proposed as an initial trigger of the self-feeding cycle that could lead to ND [6], our goal is to study the correlation between an inflammation-induced decrease in ISC synthesis on the one hand and iron accumulation on the other. It includes the characterization of structural changes of the iron compounds involved in these pathologic processes. Ferritin has been proposed as a precursor of iron accumulation in human tissue due to disturbed iron homeostasis [7]. Our *in-vitro* studies have revealed that magnetoferritin – a model system of pathological ferritin, can induce higher oxidative stress through the increased release of potentially toxic Fe^{2+} ions in the presence of vitamins C, B_2 [8] and lysosome amyloid fibrils (LAF) [9]. Moreover, the structural composition of accumulated pathological iron compounds has the potential to become a non-invasive biomarker of the early stages of ND [10].

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THE MECHANISM OF IRON-SENSING VIA THE [4FE-4S] CLUSTER OF THE RHIZOBIAL GLOBAL IRON REGULATOR RIRA

N.E. Le Brun

Centre for Molecular and Structural Biochemistry,
School of Chemistry, University of East Anglia,
Norwich Research Park, Norwich, NR4 7TJ, UK.

Iron-sulfur (Fe-S) cluster containing proteins are found widely throughout life and perform a wide range of function, including facilitating electron transfer, participating in enzymatic activity and transcriptional regulation [1] The Rrf2 family of transcriptional regulators contains a subset that employ an iron-sulfur cluster as a sensing module of cellular and environmental change through which regulation of DNA binding and transcription in coordinated [2-4]. One such example is RirA, the global iron regulator in *Rhizobium*. Work in the lab over several years has illuminated the mechanism by which this protein senses iron. It can bind a [4Fe-4S] cluster, and this form of the protein binds tightly to IRO box operator sequences upstream of RirA-regulated genes, repressing their transcription [5]. The RirA cluster is fragile and we showed that arises from the lability of one of the iron ions of the cluster, such that the following equilibrium exists: $[4Fe\text{-}4S]^{2+} \leftrightarrow [3Fe\text{-}4S]^{0} + Fe^{2+}$ that maintains the [4Fe-4S] cluster under iron-replete conditions [6]. Under low iron conditions the labile iron is readily lost, resulting in the formation of the [3Fe-4S] cluster, which is unstable to degradation. Cluster degradation, in turn, results in loss of DNA binding, leading to the de-repression of genes necessary for iron acquisition [5,6].
Here, I will discuss the iron-sensing mechanism employed by RirA, and the results of recent unpublished work aimed towards modulating the stability of the [4Fe-4S] cluster. We generated single residue substitutions of RirA that exhibited significantly enhanced cluster stability, and that retained the ability to bind IRO box DNA. This provided important insight into the likely cluster coordination in wild type RirA, and enabled predictions about the functional consequences of the residue substitution in RirA in Rhizobia. In vivo experiments enabled us to directly test those predictions and I will discuss those results here.

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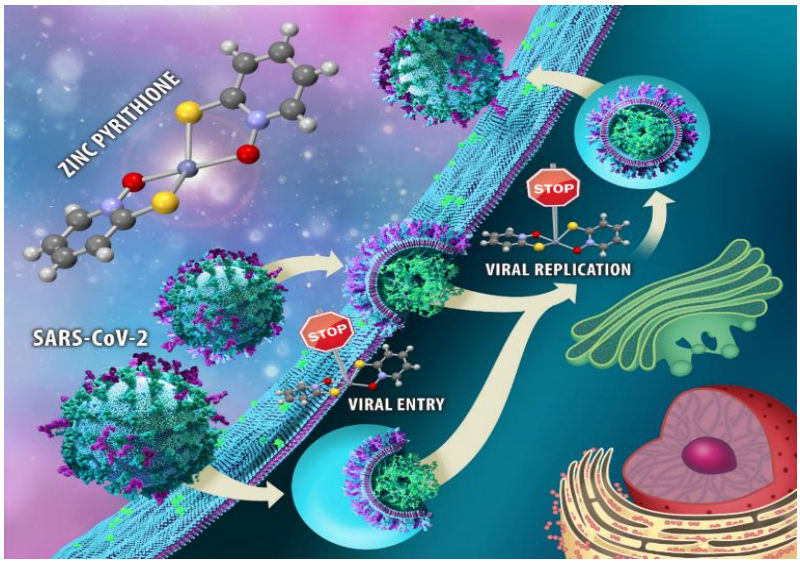
METAL-PYRITHIONE COMPLEXES, FE-S CLUSTERS AND SARS-COV-2

Jerneja Kladnik[1], Ana Dolinar[1], Jakob Kljun[1], David Perea[2], Judith Grau-Expósito[2], Meritxell Genescà[2], Marko Novinec[1], Maria J. Buzon[2,*], Iztok Turel[1,*]

[1] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia*
[2] *Infectious Diseases Department, Vall d'Hebron Research Institute (VHIR), Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, VHIR Task Force COVID-19, Barcelona, Spain*

iztok.turel@fkkt.uni-lj.si

Pyrithione is a natural compound and ionophore that easily forms complexes with metal ions. Zinc pyrithione is an established antimicrobial agent used in commercial shampoos for dandruff treatment, which exerts antifungal activity by damaging iron-sulphur clusters [1-3]. We have prepared A range of ruthenium complexes with pyrithione and analogues. These complexes inhibit variety of enzymes (aldo-keto reductases (AKRC); cholinesterases; glutathione S-trasferase; thioredoxin reductase (TrxR)…) and can be considered as multi-targeted compounds interesting for design of drugs useful for the treatment of different diseases [4-6]. In our recent work, we have studied zinc pyrithione complexes and found that such complexes are also potent inhibitors of PL_{Pro} and cathepsin L enzymes with *ex vivo* inhibition of SARS-CoV-2 entry and replication [7] (Figure). It seems such compounds hold immense potential for the development of a possible out-patient treatment for Covid-19 and further studies, especially on their mode of action are needed.



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STRUCTURE-FUNCTION RELATIONSHIPS OF RADICAL SAM ENZYMES

Yvain Nicolet

Univ. Grenoble-Alpes, CEA, CNRS,
Institut de Biologie Structurale, Metalloproteins Unit,
71 avenue des Martyrs 38044 Grenoble cedex 1 France

yvain.nicolet@ibs.fr

Radical *S*-adenosyl-L-methionine (SAM) proteins are most likely members of the widest superfamily of enzymes.[1,2] These metalloproteins reduce a [Fe_4S_4] cluster to cleave SAM into methionine and a highly reactive 5´-deoxyadenosyl radical species, which, in turn, in most cases abstracts a hydrogen atom from a given substrate, hence triggering a radical-based reaction. In this presentation we will show how X-ray crystallography can be a powerful tool to monitor reactions directly in crystals, thus giving unvaluable insights into intermediates and how the protein matrix drives and tunes these reactions. Using the radical SAM enzyme HydE involved in the assembly of the [2Fe]H subcluster of the [FeFe]-hydrogenase,[3] we were able i) to trap intermediates in the time course of FeS cluster assembly and degradation[4] and ii) to follow a radical-based reaction directly in crystals.[5]

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IRON AND IMMUNOMETABOLISM IN THE LUNG

Suzanne M. Cloonan[1,2]

[1] *School of Medicine, Trinity Biomedical Sciences Institute and Tallaght University Hospital, Trinity College Dublin, Ireland*
[2] *Division of Pulmonary and Critical Care Medicine, Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, New York, USA*

suzanne.cloonan@tcd.ie

Immunometabolism is the study of how metabolism controls immune cell function; whereby immune cells will source, utilize and re-programme metabolism to serve their functional needs. As our understanding of this rapidly emerging field expands, we are only beginning to appreciate that iron metabolism may also be essential for the correct functioning of key metabolic pathways (e.g., glycolysis, fatty acid oxidation, the tricarboxylic acid cycle, etc.) engaged by immune cells. The regulation of iron by the innate immune system is also a form of nutritional immunity which is defined as the sequestration of bioavailable trace metals such as iron by the host to limit pathogenicity by invading microorganisms. As one of the most conserved activities of the innate immune system, limiting the availability of iron by cells of the immune system serves not only to conceal this vital nutrient from invading bacteria but also operates to tightly regulate host immune cell responses and function. In the setting of chronic lung disease, the regulation of iron by the host is often disrupted, leading to the altered availability of these nutrients to commensal and invading opportunistic pathogenic microbes. Similarly, alterations in the uptake, secretion, turnover and redox activity of iron has significant repercussions for immune cell function including the response to and resolution of infection. This talk will discuss the intricate role of iron in immune cells of the lung and how changes in iron metabolism as a result of chronic lung disease may alter the airway microbiome, disease progression and the response to infection.

IMPORTANCE OF FE-S CLUSTERS FOR THE CATALYTIC FUNCTION OF [NIFE]-HYDROGENASES AND THEIR POSSIBLE ROLE IN PATHOGENESIS

Giorgio Caserta and Oliver Lenz

Institut für Chemie,
Technische Universität Berlin,
10623 Berlin, Germany

giorgio.caserta@tu-berlin.de

Hydrogenases are a diverse group of metalloenzymes that catalyze one of the simplest molecular reactions, the reversible conversion of dihydrogen (H_2) into protons and electrons [1]. Hydrogenases are widespread in nature occurring in bacteria, archaea, and some unicellular eukaryotes [2]. There is experimental evidence that even pathogenic microorganisms consume or produce H_2 using hydrogenases, contributing to their ability to persist in host tissues and in the environment [3]. The bipartite core module of [NiFe]-hydrogenases utilizes the first-row transitions metals, nickel and iron, for catalysis and consists of a large subunit bearing the catalytic $NiFe(CN)_2(CO)$ cofactor and a small subunit carrying various (in number and type) electron-transferring Fe-S clusters (**Fig. 1**) [4,5]. These Fe-S centers are crucial for the exquisite catalytic performance of these enzymes, determine the catalytic bias (H_2 oxidation *vs* H^+ reduction) and contribute to O_2 tolerance in certain members of the [NiFe]-hydrogenase family.

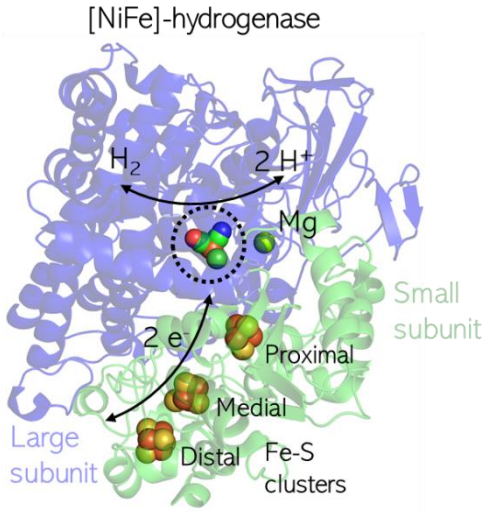

Figure 1. [NiFe]-hydrogenase core module consisting of the catalytic large subunit (blue) equipped with the heterobimetallic $NiFe(CN)_2(CO)$ active site (dashed circle) and the small subunit (green) harboring three different Fe-S clusters. Metal cofactors are shown as spheres.

In this talk I will provide an overview on the importance of Fe-S clusters in the maturation, function, and O_2 tolerance of [NiFe]-hydrogenases, focusing on enzymes from the model organism *Cupriavidus necator*. Emphasis will be placed on the biochemical/biophysical methods we currently use to investigate the involved metal sites.

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RESONANCE RAMAN SPECTROSCOPY OF FE-S CLUSTER CONTAINING PROTEINS, FROM UNKNOWN TO UNUSUAL

Smilja Todorovic

Instituto de Tecnologia Química e Biológica António Xavier,
Universidade NOVA de Lisboa,
Av. da República, 2780-157, Oeiras, Portugal

smilja@itqb.unl.pt

Resonance Raman spectroscopy (RR) provides sensitive and selective information on chromophores in proteins. Upon excitation into S → Fe ligand to metal charge transfer band, RR spectra of Fe-S containing proteins reveal vibrational fingerprint of the cluster, allowing for discrimination between different cluster types, between bridging and terminal Fe–S vibrational modes and presence of non-cysteinyl coordination [1, 2]. For these reasons, resonance Raman spectroscopy has been playing an exceptionally active role in the studies of Fe–S proteins of diverse structures and functions, as well as processes (i.e., degradation, oxidation, cluster conversion and biogenesis) and interactions that involve these specific bonds in a certain cluster [1, 2].
Here we demonstrate how RR helped us reveal the cluster type in DNA repair enzymes in which their role is unknown [3, 4], and understand unusual structures, such as the non-cubane 4Fe-4S cluster [5].

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METABOLIC REGULATION OF HUMAN T CELL FUNCTION

Anna Schurich, Katie Flaherty, Molly George

Department of Infectious Diseases,
King's College London, UK

anna.schurich@kcl.ac.uk

To generate an immune response T cells need to proliferate, increase motility and employ effector mechanisms, all of which are energetically demanding. Thus, upon activation T cells reprogram their metabolism increasing glucose metabolism via glycolysis and oxidative phosphorylation in the mitochondria. When the insult is cleared, T cell memory develops, however, in a situation of prolonged stimulation with persistent antigen, in nutrient depleted or immunosuppressive environments, CD8 T cells become functionally exhausted. T cell exhaustion is a major hurdle to the successful application of therapeutic T cells, this is especially true in the treatment of solid tumours, which provide a challenging microenvironment. We and others have shown that in situations of persistent stimulation, such as in chronic infections or in cancer, sustaining T cell responses is dependent on the maintenance of mitochondrial metabolism. However, the process of functional exhaustion is accompanied or possibly driven by loss of mitochondrial function. Many aspects of regulation of mitochondrial function in T cells and the key mechanisms leading to mitochondrial dysfunction remain to be defined.

Iron, a key component of catalytic molecules in the mitochondria, plays an important role in the systemic regulation of immune responses. We are interested in understanding the possible role of these molecules in the regulation of the T cell response and the potential for therapeutic manipulation.

MATURATION STRATEGY INFLUENCES EXPRESSION LEVELS AND COFACTOR OCCUPANCY IN FE-S PROTEINS

Ingrid Span[1], Melissa Jansing[2], Steffen Mielenbrink[2], Hannah Rosenbach[2], Sabine Metzger[3]

[1] *Bioanorganische Chemie, Department Chemie und Pharmazie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany;*
[2] *Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany;*
[3] *MS-Platform Biocenter, Cluster of Excellence on Plant Science (CEPLAS), University of Cologne, Zülpicher Strasse 47b, 50674 Cologne, Germany*

ingrid.span@fau.de

Iron-sulfur clusters are ubiquitous cofactors required for fundamental biological processes. Structural and spectroscopic analysis of Fe-S proteins is often limited by low cluster occupancy in recombinantly produced proteins. In this work, we report a systematic comparison of different maturation strategies for the three well-established [4Fe-4S] proteins. Aconitase B, HMBPP reductase (IspH), and quinolinate synthase (NadA) were used as model proteins as they have previously been characterized. The protein production strategies include expression of the gene of interest in BL21(DE3) cells, maturation of the apo protein using chemical or semi-enzymatic reconstitution, co-expression with two different plasmids containing the iron-sulfur cluster (*isc*) or sulfur formation (*suf*) operon, a cell strain lacking IscR, the transcriptional regulator of the ISC machinery, and an engineered “SufFeScient” derivative of BL21(DE3). Our results show that co-expression of a Fe-S biogenesis pathway influences the protein yield and the cluster content of the proteins. The presence of the Fe-S cluster is contributing to correct folding and structural stability of the proteins. In vivo maturation reduces the formation of Fe-S aggregates, which occur frequently when performing chemical reconstitution. Proteins show a distinct preference for either the ISC or the SUF pathway, which can be related to their role in the cell. Furthermore, we show that the in vivo strategies can be extended to the radical SAM protein ThnB, which was previously only maturated by chemical reconstitution. Our results shed light on the differences of in vitro and in vivo Fe-S cluster maturation and points out the pitfalls of chemical reconstitution.

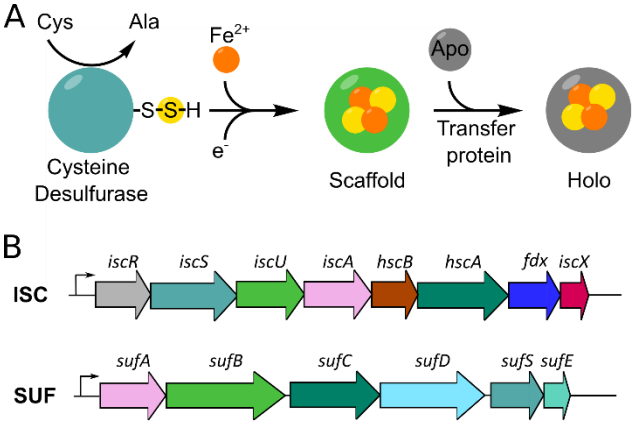

Figure 1. Iron-sulfur cluster biosynthesis in *Escherichia coli*. A) Simplified representation of Fe-S cluster biogenesis. B) Overview of the ISC and SUF machinery producing gene clusters from *E. coli*. Each operon contains proteins with similar functions, which are shown in the same color.

COMPUTATIONAL STUDIES OF FES CLUSTER CONTAINING PROTEIN DCCP

Maria Andrea Mroginski, ,Nadia Elghobashi-Meinhardt

Institut für Chemie,
Technische Universität Berlin,
10623 Berlin, Germany

andrea.mroginski@tu-berlin.de

FeS clusters containing proteins are involved in many fundamental biological processes. In particular, they play a fundamental role in catalyzing a redox reactions essential for carbon, oxygen, hydrogen and nitrogen metabolism. These proteins harbor at least one iron-sulfur cluster cofactor which are commonly found in nature as [2Fe2S] and [4Fe4S]. These highly coupled spin systems require a multi-determinate-based approach to describe properly their complex electronic structure. A computational cheaper alternative is the Broken-Symmetry Density Functional Theory (BS-DFT) which provides a single-determinate broken symmetry solution to the multi-determinate problem [1]. In this presentation I will report on the hybrid BS-DFT/MM (molecular mechanics) calculations on the double cubane cluster of DCCP (Double Cubane Cluster Protein) [2]. This recently discovered enzyme catalyzes ATP driven reduction of small molecules, otherwise only reported for nitrogenase [3]. Emphasis will be given to the characterization of spin states and structural parameters of double cubane cluster.

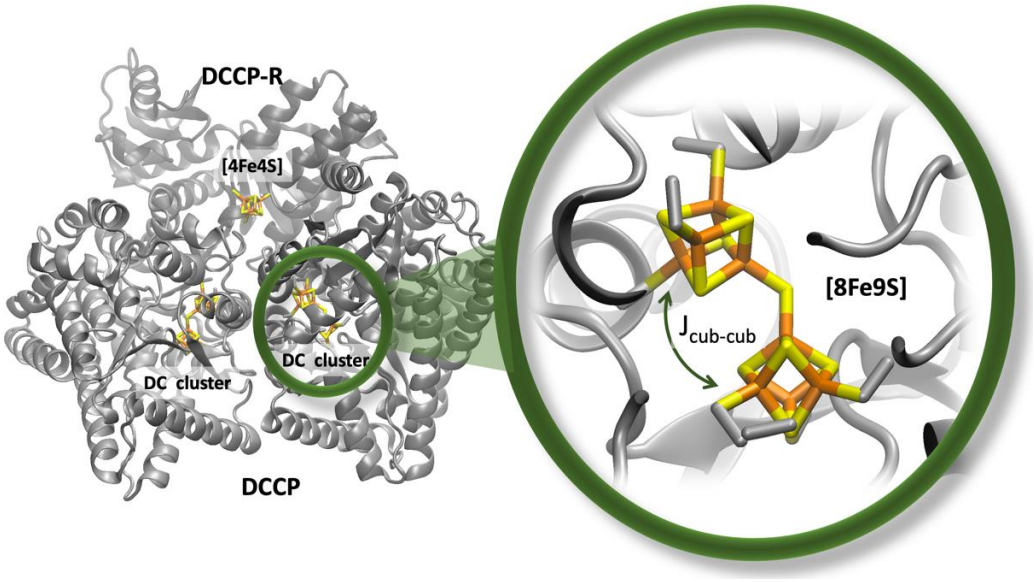

Figure 1: Overall structure of DCCP: DCCP-R complex (left) and close-up view of the [8Fe9S] cluster binding site in the DCC (right).

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FUNCTIONAL ANALYSES OF VIPERIN AND LIPOYL SYNTHASE HIGHLIGHT CELLULAR REQUIREMENTS FOR THE ACTIVITY OF RADICAL SAM ENZYMES

Oliver Stehling[1,2], Vinzent Schulz[1,2], Arunkumar S. Upadhyay[3], Roland Lill[1,2]

[1] *Institut für Zytobiologie, Philipps-Universität, Karl-von-Frisch-Str. 14, Marburg, Germany;*
[2] *Zentrum für Synthetische Mikrobiologie, Karl-von-Frisch-Str. 14, Marburg, Germany;*
[3] *Department of Clinical Microbiology, Umeå University, Sweden*

stehling@staff.uni-marburg.de

Radical SAM enzymes contain [4Fe-4S] cofactors and catalyse chemically challenging reactions by reductive cleavage of S-adenosylmethionine (SAM). Viperin (virus-inhibitory protein, endoplasmic reticulum-associated, interferon-inducible) is a type I IFN-inducible radical SAM enzyme which exhibits antiviral activity against a broad spectrum of RNA viruses by converting cytidine triphosphate (CTP) into 3'-deoxy-3',4'-didehydro-CTP (ddhCTP), causing the premature termination of RNA synthesis catalysed by viral RNA-dependent RNA polymerases. Viperin's antiviral function is strictly dependent on [4Fe-4S] cofactor insertion mediated by the cytosolic-nuclear iron-sulphur (Fe/S) protein assembly (CIA) system. This system uses an ill-defined, mitochondria-derived substrate to assemble a [4Fe-4S] cluster on the NBP35-CFD1 scaffold complex. Subsequently the Fe/S protein CIAO3 assists transfer of the cluster from NBP35-CFD1 to the CIA targeting complex (CTC) comprising CIAO1, CIAO2B, and MMS19. These proteins operate in varying combinations to mediate client protein-specific insertion of the metallocofactor. We performed RNAi and overexpression studies and identified CIAO1 and CIAO2B as critical CTC components assisting the maturation of viperin. Mutational analyses revealed that viperin establishes physical contact to the CTC by a conserved C-terminal tryptophan residue which is essential for cofactor insertion and viperin function in vivo. While the [4Fe-4S] cofactor holds a key role in SAM reduction, the physiological electron donor that stimulates viperin's antiviral activity is not known yet. Functional analysis of a different radical SAM enzyme, mitochondrial lipoyl synthase (LIAS), highlighted the critical role of physiological electron supply for proper radical SAM activity. LIAS contains two [4Fe–4S] clusters, one participating in SAM cleavage, and the other serving as a sulphur source for conversion of the octanoyl substrate into the lipoyl cofactor product. Using RNAi and CRISPR-Cas approaches in human tissue culture we depleted the mitochondrial ferredoxins FDX1 and FDX2 individually, and studied the knock-down/knock-out phenotypes. Both ferredoxins were found to be involved in LIAS activation yet performing mutually exclusive roles. Loss of FDX2 affected integrity and function of mitochondrial Fe/S proteins including LIAS, in line with the function of FDX2 in mitochondrial Fe/S cluster assembly. In contrast, we did not detect any involvement of FDX1 in Fe/S cluster formation. Reconstitution of human lipoyl synthesis by adapting an established bacterial in vitro system revealed that FDX1 serves as the dedicated electron donor to kickstart the radical SAM-dependent LIAS reaction. Strikingly, FDX1 was more potent than the artificial strong reductant dithionite, and could not efficiently be replaced by FDX2. Our findings establish a framework of cellular requirements for activation of two distinct radical SAM enzymes. The identification of the still elusive physiological electron donor of viperin will contribute to our understanding of its broad antiviral activity.

P1. COMPUTATIONAL APPROACHES FOR RATIONAL DESIGN OF NEW INHIBITORS OF FES CLUSTER PROTEINS

Amalia STEFANIU, Misu MOSCOVICI, Lucia PINTILIE

National Institute of Chemical,
Pharmaceutical Research and Development, Bucharest,
112 Vitan Av., 3rd District, 031299 Bucharest, Romania

astefaniu@gmail.com

The aim of this work was to evaluate the potential of a small-molecule to modulate Fe-S cluster assembly. Previous studies [1,2] revealed the identification of a small ligand (Figure 1) able to inhibit the fermentative growth by Iron-Sulfur cluster assembly inhibition. The investigated structure was proved to regulate the virulence and the metabolism in *Staphylococcus aureus* [2]. In the discovery process of novel therapeutic targets for handling the issues of antimicrobial resistance, *in silico* approaches, such as prediction of drug-like properties using hybrid algorithms based on Density Functional Theory and docking simulations aiming to evaluate molecular interactions between target and potential ligand candidates, are often used with reliable and accurate results. Iron is crucial to the infectious life cycle of pathogens and it is incorporated into proteins as Fe-S cluster cofactors, required for a variety of cellular processes. Thus, inhibiting the function of Fe-S cluster-dependent processes, represents an intriguing option.
In the present study, B3LYP/DFT/6-31G (d, p) method [3] was employed to assess molecular and QSAR properties of the investigated ligand. Further, its lowest energy conformer was used in docking simulations against SufS protein from *Staphylococcus aureus* [4].

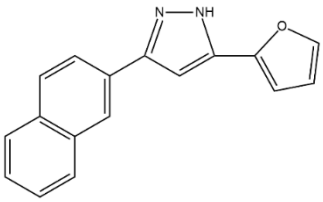

Figure 1. Structure of 5-(furan-2-yl)-3-(naphthalen-2-yl)-1H-pyrazole

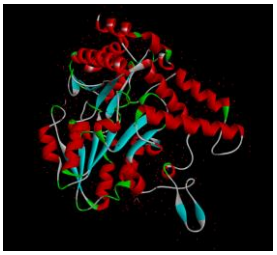

Figure 2. SufS from *Staphylococcus aureus*, PDB ID: 8D8S

Observed findings are useful to predict the occurring complexing interactions, the best binding pose and affinity, respectively. Results are discussed in term of hydrogen bond interactions, docking score and RSMD.
Choosing the cysteine desulfurase SufS, which is involved in iron-sulfur (Fe-S) cluster biogenesis pathway, to serve as a therapeutic target, could be a successful attempt to identify new therapeutic strategies for treating infections caused by *S. aureus* by inhibiting the Suf-like pathway.

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P2. DFT AND QTAIM STUDIES ON FeS CLUSTERS: STRUCTURE AND BONDING PROPERTIES

Avni Berisha

Department of Chemistry, Faculty of Natural and Mathematics Science,
University of Prishtina, 10000 Prishtina, Kosovo

avni.berisha@uni-pr.edu

Metalloproteins contain Iron–Sulfur (Fe-S) clusters, which are sulfide-linked (di-), (tri-)iron, or tetra-iron clusters. Iron (Fe) is a transition metal capable of forming cations with incomplete d subshells. This attribute confers variable valency (II or III) and the ability to form coordination units, such as Fe-S clusters, on Fe. The redox potential of Fe-containing cofactors can range between 650 and +450 mV depending on their ligands, chemical structures, and protein folds [1]. Due to the fluctuating redox potential of Fe, Fe-S clusters can transfer electrons, particularly when they are placed sequentially with individual distances of 14 Å [2].

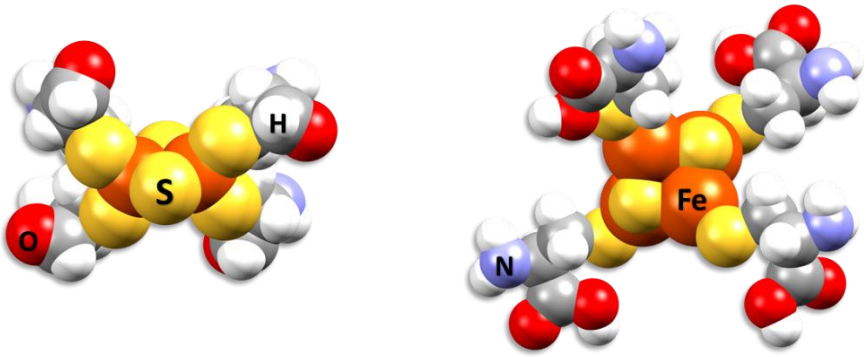

Figure 1. Optimized geometry for the of studied FeS clusters: a) classic 2Fe-2S coordinated by four cysteine (Cys) residues and b) 4Fe-4S coordinated by four Cys residues.

Common types of Fe-S clusters (Figure 1) include: (1) classic 2Fe-2S coordinated by four cysteine (Cys) residues; (2) NEET-type 2Fe-2S coordinated by three Cys and one His residue; (3) Rieske-type 2Fe-2S coordinated by two Cys and two His residues; (4) 3Fe-4S coordinate by three Cys residues and (5) 4Fe-4S coordinated by four Cys residues (or three Cys).
In this study, utilizing DFT simulations, a comprehensive investigation of structure, interaction, and bonding is carried out on two typical FeS clusters, as shown in Figure 1.

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P3. MULTIPLE MITOCHONDRIAL DYSFUNCTIONS SYNDROME 2: STRUCTURAL CONSEQUENCES OF THE H96R BOLA3 MUTATION

Beatrice Bargagna[1,3], Francesca Camponeschi[1,3], Lucia Banci[1,2,3].

[1] Magnetic Resonance Center-CERM, University of Florence, Via Luigi Sacconi 6, 50019, Sesto Fiorentino, Florence, Italy
[2] Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP) Via L. Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy
[3] Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy

E-mail: *bargagna@cerm.unifi.it*

Iron-sulfur (Fe/S) clusters are simple inorganic protein cofactors composed by ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron and sulfide (S^{2-}) ions. They are ancient, ubiquitous prosthetic groups, supposed to be involved in the development of the very first forms of life. Different biogenesis machineries guarantee the clusters biosynthesis and incorporation into scaffold proteins, to obtain their final holo forms [1-2]. The Fe/S clusters biosynthesis starts in the mitochondrion, where Fe/S proteins take part to important cellular pathways such as oxidative phosphorylation, lipoic acid synthesis, and iron metabolism. Fe/S proteins contribute to the main function of this organelle, which is responsible for the production of cellular energy. Mutations in genes encoding for some components of the mitochondrial ISC machinery, namely NFU1, BOLA3, IBA57, ISCA2 and ISCA1 proteins, are related to a group of rare syndromes which cause autosomal recessive diseases, and which have been identified, since 2011, as Multiple Mitochondrial Dysfunctions Syndromes (MMDS) types 1 to 5. The main characteristic of these rare neurodegenerative disorders is a lower energy metabolism, with consequent impairment in neurologic development, muscle weakness, lactic acidosis, respiratory failure.
Bi-allelic mutations in the gene encoding for BOLA3 cause MMDS type 2, which typically appears in early childhood with encephalopathy, leukodystrophy, lactic acidosis, nonketotic hyperglycinemia, cardiomyopathy, and death [3-4]. A homozygous H96R (c. 287 A > G) variant was identified in the BOLA3 gene, on a fully conserved residue of the bola-like protein family, that is involved in the coordination of the [2Fe-2S] cluster in the [2Fe-2S]-GLRX5-BOLA3-GS_2 heterocomplex. The mutation is responsible for severe lactic acidosis and combined respiratory chain complex deficiencies, implication of a wide range of organs, and hypertrophic cardiomyopathy. The molecular basis of the pathogenicity of the H96R mutation have not been investigated yet [5-6].
The investigation of the structural consequences of BOLA3 mutation, and its impact on the protein interaction with its partner monothiol glutaredoxin GLRX5 in the formation of [2Fe-2S]-GLRX5-BOLA3-GS_2 complex, will be presented and discussed.

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P4. BACTERIAL FRATAXIN: WHEN EVOLUTION MEETS GENETICS

Béatrice Roche[1], Yohann Duverger[1], Pierre Simon Garcia[2], Siyi Liu[1], Julien Pérard[3], Sandrine Ollagnier de Choudens[3], Frédéric Barras[1] and Béatrice Py[1]

[1] CNRS, Aix-Marseille Université, Laboratoire de Chimie Bactérienne UMR 7283, Institut de Microbiologie de la Méditerranée, Institut Microbiologie Bioénergies Biotechnologie, Marseille, France
[2] Institut Pasteur, Department of Microbiology, Unit Stress, Adaptation and Metabolism in Enterobacteria, Université Paris Cité, UMR CNRS 6047, Paris, France
[3] Univ. Grenoble Alpes, CNRS, CEA, Laboratoire de Chimie et Biologie des Métaux, F-38000 Grenoble, France

py@imm.cnrs.fr

Among central players involved in Fe-S cluster biogenesis, the protein called Frataxin (FXN) is still the subject of intense investigation. In eukaryotes, FXN deficiency leads to severe defects in Fe-S cluster formation, and in humans, is responsible for the neurodegenerative disease Friedreich's ataxia. In contrast, a defect in the bacterial FXN homolog, CyaY, leads to mild phenotypes in *E. coli*. This apparent low importance of CyaY is has been proposed to be driven by the nature of a single amino acid in the Fe-S scaffold protein, IscU. Indeed, change of the prokaryotic amino acid, Ile108, to eukaryotic amino acid Met, turned *E. coli* into a CyaY-dependent organism. Here, we reported the occurrence of spontaneous suppressor mutants of the *iscU*$_{M108}$ Δ*suf* Δ*cyaY* strain. The suppressors exhibited a single amino acid substitution, which changes the Met$_{108}$ residue in IscU into a Val residue. Such mutation was able to suppress growth defect of the original parental strain when grown in stress condition (iron limitation, oxidative stress), and in minimal medium. Also, when compared to the original parental strain activity of our reporter Fe-S proteins was restored. Detailed *in vitro* biochemical analyses to characterize the IscU$_{V108}$ scaffold protein, indicated that in contrast to IscU$_{M108}$, IscU$_{V108}$ behaves like wt IscU $_{I108}$ for IscS binding and rate of Fe-S cluster formation. By assessing maturation of several Fe-S cluster-containing proteins, we showed that in *E. coli* containing the IscU$_{V108}$ scaffold, CyaY is dispensable for Fe-S cluster biogenesis. Very interestingly, this result is an experimental validation of our previous bioinformatic analyses evidencing that prokaryotic species lacking CyaY mostly contain a Val residue at position 108 in the IscU protein. Exploiting the prokaryotic diversity, we replaced *E. coli iscU* by a naturally occurring Val$_{108}$ containing-IscU from *Magnetococcus marinus* (IscU$_{V108_Mm}$) and showed that the ISC machinery was functioning in a fully CyaY-independent manner. Last, when residue 108 was substituted by a methionine, the single IscU variant from *M. marinus*, CyaY was able to boost functioning of the ISC machinery. Altogether, these data further demonstrated the key role of the IscU scaffold protein, and especially the 108th position, for FXN to exert its positive role. Our results remarkably showed that genetic approaches can mimic what was pointed out by evolutionary studies.

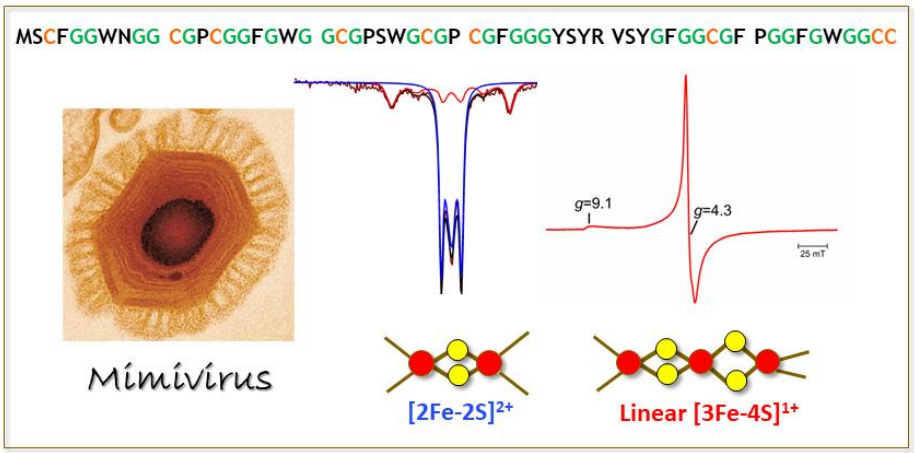
P5. A UNIQUE FAMILY OF GLYCINE/CYSTEINE-RICH IRON-SULFUR PROTEINS IN MEGAVIRINAE GIANT VIRUSES

Alejandro Villalta[1], Batoul Srour[2], Audrey Lartigue[1], Martin Clémancey[3], Bruno Guigliarelli[2], Geneviève Blondin[3], Chantal Abergel[1], Bénédicte Burlat[2]

[1] *Aix-Marseille Univ, CNRS, Information Génomique et Structurale (IGS), Marseille, France.*
[2] *Aix-Marseille Univ, CNRS, Bioénergétique et Ingénierie des Protéines (BIP), Marseille, France.*
[3] *Univ Grenoble Alpes, CNRS, CEA, Laboratoire de Chimie et Biologie des Métaux (LCBM), Grenoble, France.*

bburlat@imm.cnrs.fr

Giant viruses are nucleocytoplasmic large DNA viruses (NCLDVs)[1] with up to 2.8 Mb genomes encoding 1500 proteins, most of them without resemblance with other cellular or viral proteins.[2, 3] Giant viruses are found in various habitats and infect eukaryotes, mostly protists and microalgae.[4] In the giant virus mimivirus, we have discovered a protein with an amino acid composition exceptionally rich in glycine and cysteine residues. This small 6 kDa protein is among the most abundant proteins in the icosahedral 0.75 µm viral particles, it has no predicted function but is probably essential for infection. UV/vis, EPR, and Mössbauer studies revealed that the viral protein, coined GciS (Glycine/Cysteine-rich Iron-Sulfur protein), accommodated two distinct Fe-S clusters: a diamagnetic S=0 $[2Fe\text{-}2S]^{2+}$ cluster and a paramagnetic S=5/2 linear $[3Fe\text{-}4S]^{1+}$ cluster, a geometry rarely stabilized in native proteins. [5] Orthologs of mimivirus GciS were identified within all clades of *Megavirinae*, a *Mimiviridae* sub-family infecting *Acanthamoeba*, and displayed the same spectroscopic features. Thus, these glycine/cysteine-rich proteins form a new family of viral Fe-S proteins sharing unique Fe-S cluster binding properties. These structural features may be clues to discovering novel functions for viral Fe-S proteins during infection.



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P6. FeS CLUSTER COORDINATING GLUTAREDOXINS

Carsten Berndt[1] and Christopher Horst Lillig[2] et al.

[1] *Heinrich-Heine Universität, Department of Neurology, Düsseldorf, Germany*
[2] *Universitätsmedizin Greifswald, Institute of Medical Biochemistry and Molecular Biology, Greifswald, Germany*

Glutaredoxins (Grxs) are present in all kingdoms of life. In the mammalian system, four Grxs are localized in cytosol/nucleus and mitochondria. These Grxs regulate either redox or iron homeostasis. Grxs 1 and 2 are key enzymes in redox signaling regulating reversible oxidative posttranslational cysteine modifications, wheres Grxs 3 and 5 are essential for iron trafficking and FeS cluster biogenesis. Grxs 2, 3, and 5 were characterized as FeS-proteins coordinating one or two of these cofactors in a dimer. All FeS-Grxs are unique FeS-proteins as they use the cysteine residues of non-covalently bound glutathione molecules as non-protein ligands in addition to the active site cysteines.

Only five amino acids shortly before the active site/the FeS coordination site determine the activity of FeS-Grxs as oxidoreductase or as FeS cluster transferase. These amino acids form a loop affecting the glutathine binding and the stability of the bound FeS cluster. Less stable cluster coordination allows Grx5 to transfer its bound cofocator. Exchange of the loop forming amino acids exchanged also the functions of the proteins. The role of FeS cluster coordination in oxidoreductases is not fully understood, especially since the coordination inhibits the active site. However, the FeS cluster seems to be important during neuroinflammation. Only Grx2 with the ability to coordinate the FeS cluster protects the myelin structure. Myelin damage is induced by nitric oxide secreted by activated microglia. The FeS cluster is destroyed by nitric oxide and detoxicates it via the formation of diglutathionyl dinitrosyl iron complexes.

Our research is interested in the identification of further FeS-depending Grx functions and the (de-) activation of Grxs by FeS cluster coordination.

P7. A BIOPHYSICAL VIEW OF THE FE-S CLUSTERS IN THE DNA REPAIR ENZYMES HNTH1 AND UVRC

Catarina Barbosa[1], Célia M. Silveira[1], Filipe Rollo[1], Lidia Zuccarello[1], Elin Moe[1], Joanna Timmins[2] and Smilja Todorovic[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República, 2780-157, Oeiras, Portugal;
[2] Univ. Grenoble Alpes, CEA, CNRS, IBS, F-38000, Grenoble, France.

catarina.barbosa@itqb.unl.pt / celiasilveira@itqb.unl.pt

Iron sulfur clusters have been identified in a variety of DNA repair enzymes that are essential for genome maintenance. The cofactors are typically not directly involved with the catalytic activity, which poses questions about their roles in DNA repair enzymes [1]. In this work, we report the biophysical characterization of two endonucleases that harbor FeS clusters: the human endonuclease III, hNTH1, from the Base Excision Repair (BER) pathway and UvrC from *Deinococcus radiodurans* (DvUvrC), which takes part in bacterial Nucleotide Excision Repair (NER) pathway. Resonance Raman (RR) spectra of hNTH1 reveal the presence of a fully cysteinyl coordinated [4Fe–4S] cluster, as observed in bacterial endonucleases III (EndoIII) [2]. The RR spectra of DrUvrC indicate the fingerprint of a [3Fe–4S] cluster. This is in agreement with the presence of only three instead of four conserved cysteine residues in the cysteine-rich motif that most commonly coordinates the [4Fe–4S] clusters (e.g. in the UvrC from *E. coli*, EcUvrC) [3]. The redox processes of the two enzymes were evaluated by cyclic voltammetry upon immobilization onto biocompatible Au electrodes modified with DNA- and/or MUA-terminated SAMs. Adsorption of hNTH1 and UvrC to the modified electrodes was probed by surface enhanced absorption infrared (SEIRA) and surface enhanced Resonance Raman (SERR) spectroscopies. UvrC displays a redox transition at $E^{0'}$ 70 mV upon binding to negatively charged MUA surfaces, which suggests the redox activity of the cofactor is not DNA dependent. Full-length hNTH1 and its truncated form (hNTH1Δ89, that lacks the N-terminal domain composed of the first 89 amino acids, and is structurally highly analogous to bacterial enzymes), were shown to have distinct DNA binding properties. This suggests that the N-terminal domain of hNTH1, which is absent in bacterial EndoIIIs may be important for damage recognition and DNA binding [2]. We furthermore demonstrate that vibrational spectroelectrochemistry provides new insights into the mechanistic models of hNTH1 and UvrC endonucleases.

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P8. A DIVERGED TRANSCRIPTIONAL NETWORK FOR USAGE OF TWO FE-S CLUSTER BIOGENESIS MACHINERIES IN THE DELTA-PROTEOBACTERIUM *MYXOCOCCUS XANTHUS*

Mathieu Sourice[1], Isabel Askenasy[2], Pierre Simon Garcia[3,4], Yann Denis[5], Gaël Brasseur[1], Patricia J. Kiley[2], Béatrice Py[1], Corinne Aubert[1].

[1] LCB, IMM, IM2B, CNRS; Aix-Marseille Université, Marseille France; [2] Department of Biomolecular Chemistry, University of Wisconsin-Madison, Madison, Wisconsin, USA; [3] Department of Microbiology, Unit Stress Adaptation and Metabolism in Enterobacteria, Institut Pasteur, Université Paris Cité, Paris, France; [4] Department of Microbiology, Unit Evolutionary Biology of the Microbial Cell, Institut Pasteur, Université Paris Cité, Paris, France; [5] Plate-forme Transcriptomique, IMM, CNRS; Aix-Marseille Université, Marseille France.

aubert@imm.cnrs.fr

Fe-S clusters are essential cofactors for most organisms and control a wide variety of key biological processes. Therefore, maintaining Fe-S cluster homoeostasis is an essential task for all organisms. *In vivo*, biogenesis of Fe-S clusters is ensured by multiprotein machineries ISC and SUF. The well-studied model bacteria *E. coli,* uses both machineries to achieve this task with ISC the "housekeeping" machinery while SUF is dedicated to stress conditions [1].
In this study, we show that *Myxococcus xanthus*, a Deltaproteobacterium phylogenetically distant from Enterobacteria, evolved an independent transcriptional scheme to use ISC and SUF machineries concomitantly [2]. The transcriptional response in *M. xanthus* is directed by RisR, a Rrf2-regulator, phylogenetically distant from IscR that regulates *isc* and *suf* operons in *E. coli*. RisR seems to harbor an 4Fe-4S cluster and holo-RisR represses both *isc* and *suf* operons. We further show that unlike what has been found in most prokaryotes, the ISC and SUF machineries appear to be fully interchangeable in maintaining housekeeping levels of Fe-S biogenesis and in synthesizing the Fe-S cluster of RisR regulator. In response to oxidative stress and iron starvation, RisR in its apo-form is not anymore able to repress *isc* an *suf* operons. Netherless, like in *E. coli*, the contribution of the SUF machinery is greater than the ISC machinery in stress conditions.
Altogether, these findings shed light on the diversity of homeostatic mechanisms exploited by bacteria to coordinate and enable use the two Fe-S cluster biogenesis. This new knowledge also paves the way to improve production of Fe-S dependent secondary metabolites using *M. xanthus* as a chassis.

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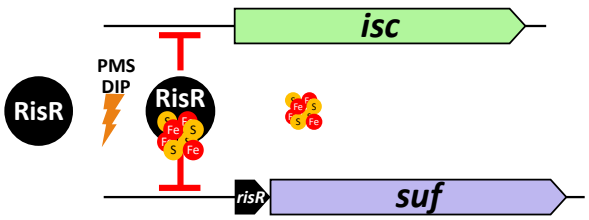

Model of regulation of *isc* and *suf* operons in *Myxococcus xanthus*.

P9. RELAXATION-BASED NMR ASSIGNMENT: SPOTLIGHTS ON LIGAND BINDING SITES IN HUMAN CISD3

Deborah Grifagni[1], José M Silva[1], Francesca Cantini[1,2,3], Mario Piccioli[1,2,3], Lucia Banci[1,2,3]

[1] Magnetic Resonance Center (CERM), University of Florence, via Sacconi 6, Sesto Fiorentino, 50019, Italy
[2] Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, Sesto Fiorentino, 50019, Italy c
[3] Consorzio Interuniversitario Risonanze Magnetiche MetalloProteine (CIRMMP), via Sacconi 6, Sesto Fiorentino, 50019, Italy

grifagni@cerm.unifi.it

The complete NMR resonance assignment of a biological macromolecule is mandatory to monitor site-specific interactions with drugs and/or with other biomolecules at atomic level in solution. Iron sulfur proteins offer many interesting case studies to address the feasibility of an extended/complete NMR assignment in the presence of paramagnetic, fast relaxing centers, such as magnetically coupled high spin $Fe^{3+/2+}$ ions. NEET proteins climbed the "top of the pop" of metalloproteins due to their possible role in reactive oxygen species and iron homeostasis regulations [1, 2]. It has been also reported that they are overexpressed in a wide variety of cancer types and are potential targets for diabetes treatment [3, 4]. In humans, this family has 3 members: mitoNEET, CISD2 and CISD3. At variance with the other human NEET proteins, CISD3 contains two CDGSH cluster-binding motifs within a single polypeptide chain that folds as a monomer in solution [5]. Here, by the case of CISD3, we provide evidence that paramagnetic relaxation can be used as a tool to revive signals of the cluster environment and to improve the sequence specific assignment of the protein.
This methodology allowed us to characterize the interactions of NEET proteins with protein partners and drug candidates as well as to identify the residues which are the key players in the biological function of this protein family [6].

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P10. SYNTHESIS, MOLECULAR DOCKING, MOLECULAR DYNAMIC SIMULATION, DFT CALCULATION, AND ANTIMICROBIAL ACTIVITY OF NOVEL COUMARIN DERIVATIVES

Demokrat Nuha [1,2,3,], Asaf Evrim Evren [1,4], Özge Kapusiz [5], Nalan Gundogdu-Karaburun [1], Ahmet Çagri Karaburun [1], Ülküye Dudu Gül [6], and Halil Berber [2]

[1] Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470, Eskişehir, Turkey.[2] Eskisehir Technical University, Faculty of Science, Department of Chemistry, 26555, Eskişehir, Turkey.[3] University of Business and Technology, Faculty of Pharmacy, Prishtina, Kosovo.[4] Bilecik Seyh Edebali University, Vocational School of Health Services, Department of Pharmacy Services, 11000, Bilecik, Turkey.[5] Bilecik Seyh Edebali University, Graduate School of Education Sciences, Department of Bioengineering, 11230, Bilecik, Turkey.[6] Bilecik Seyh Edebali University, Faculty of Engineering, Department of Bioengineering, 11230, Bilecik, Turkey.

demokratnuha@gmail.com, demokrat.nuha@ubt-uni.net.

Since their discovery in 1929, antibiotics have undoubtedly been one of the most important and useful discoveries in medical history. Their use allows humans to survive pathogen-related disorders [1]. The World Health Organization (WHO) has highlighted antimicrobial resistance as one of the most pressing global health challenges of the twenty-first century. According to the Centers for Disease Control and Prevention (CDC), antibiotic resistance kills 700,000 people each year, with 10 million deaths predicted by 2050 if present trends continue. As a result, medicinal chemists are investigating newly developed, more potent antibacterial drugs [2].

Coumarins are well-known chemical entities with distinct properties such as simple structure, high solubility, good bioavailability, and low toxicity. Coumarin derivatives have been shown to have a variety of biological and pharmacological actions, including antibacterial activity, antifungal activity, antioxidant activity, antitumor activity, anticancer activity, anti-inflammatory activity, anticoagulant activity, antihypertensive activity, antitubercular activity, and antidepressant activity [3].

A unique series of coumarin derivatives have been synthesized, and their chemical structures have been determined using ^{1}H-NMR, ^{13}C-NMR, and LC/MS-IT-TOF spectral data. The minimum inhibitory concentration (MIC) of each compound was determined after testing it on eight bacteria and three fungus species. Some of the chemicals were effective against microbial strains. Compound **4e** was shown to be 2- and 4-fold more active (MIC: 0.97 µg/mL) against *C. parapsilopsis* than the conventional medicines voriconazole and fluconazole, respectively. Compounds **4a** and **4i** have also been shown to be effective against *E. faecalis* and *E. coli*, respectively.

Compounds **4e** and **4a** were employed in molecular docking and dynamic modeling to investigate the action mechanism of those active compounds, as well as to explain the structure-activity link. Furthermore, DFT was used to study the chemical reactivity properties of all molecules. According to the DFT calculations, the potential most active molecule is **4a**, which really exhibits higher antibacterial activity than the others.

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P11. PROBING A KEY SEMIQUINONE INTERMEDIATE IN THE MECHANISM OF RESPIRATORY COMPLEX I WITH EPR SPECTROSCOPY

Eleanor Clifford[1], John J. Wright[2], Alberto Collauto[1], Judy Hirst[2], Maxie M. Roessler[1]

[1] Department of Chemistry, Imperial College London, Molecular Sciences Research Hub, London, UK; [2] Medical Research Council Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Cambridge, UK.

e.clifford22@imperial.ac.uk

Respiratory complex I (R-CI) is fundamental to aerobic respiration and its dysfunction is involved in many human mitochondrial disorders, such as Leber's hereditary optic neuropathy and Leigh syndrome. R-CI uses a chain of seven iron–sulfur (FeS) clusters to catalyse NADH oxidation and ubiquinone-10 reduction and couples the free energy from this process to proton pumping across the inner mitochondrial membrane, contributing to the proton motive force required for ATP synthesis. It has been suggested that a semiquinone (SQ) intermediate formed during ubiquinone-10 reduction is key to this coupling mechanism, and there have been several reports of such a *g* ~ 2 species observed using electron paramagnetic resonance spectroscopy (EPR).[1] However, the weak *g* ~ 2 signal intensity and the use of membrane systems containing several components of the respiratory electron transfer chain that lead to g ~ 2 signals has, to date, prevented unambiguous assignment of observed EPR signals to SQ from R-CI.

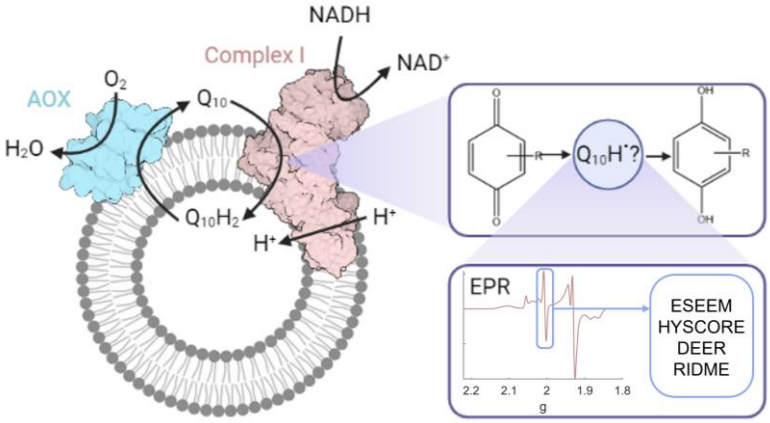

Figure 1. Continuous wave and pulsed EPR are used to investigate the properties and environment of a species giving rise to a g ~ 2 signal generated under sustained R-CI turnover.

In this work, a synthetic membrane (liposomes) system and a highly sensitive EPR set-up using a cryogenic low-noise preamplifier [3] are employed to selectively investigate an organic radical species generated during R-CI catalysis (Fig. 1). R-CI from *Bos taurus* heart mitochondria was reconstituted in to liposomes containing ubiquinone-10 and the quinol-oxidizing enzyme alternative oxidase (AOX) to ensure sustained Q_{10} turnover, and proteoliposome samples were flash-frozen under turnover. Continuous wave EPR measurements reveal the presence of a piericidin A (R-CI inhibitor) sensitive radical signal at *g* ~ 2. To ascertain whether this EPR signal can be attributed to a R-CI SQ, the properties, environment and location of the radical is probed using pulsed EPR techniques. Besides aiming to resolve a key step in the mechanism of R-CI, this work showcases how pulse EPR may be used to investigate a *g* ~ 2 species that cannot be pinpointed using continuous wave EPR alone.

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P12.[4Fe-4S] CLUSTER ASSEMBLY IN THE HUMAN CYTOSOL: THE SYNERGIC ROLE OF ANAMORSIN AND MONOTHIOL GLUTAREDOXIN-3 IN THE MATURATION OF NUBP1.

Francesca Camponeschi[1,2,3], Beatrice Bargagna[1,2,3], Sara Matteucci[2,3], Simone-Ciofi-Baffoni[1,2,3], Lucia Banci[1,2,3]

[1] Department of Chemistry, University of Florence; [2] Magnetic Resonance Center – CERM, University of Florence; [3] Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine – CIRMMP, University of Florence

camponeschi@cerm.unifi.it

The assembly of $[4Fe\text{-}4S]^{2+}$ clusters in the human cytosol, as well as in mitochondria, occurs through the reductive coupling of two $[2Fe\text{-}2S]^{2+}$ clusters [1-3]. This process requires a [2Fe-2S] cluster chaperone that donates two $[2Fe\text{-}2S]^{2+}$ clusters and an electron donor providing two electrons, but the mechanism of the assembly of $[4Fe\text{-}4S]^{2+}$ clusters in cytosol is still elusive. We previously investigated the mechanism for the formation of the N-terminal [4Fe-4S] cluster of the human P-loop NTPase NUBP1, an essential early component of the cytosolic iron-sulfur assembly (CIA) machinery. Specifically, we showed that two $[2Fe\text{-}2S]^{2+}$ clusters, donated by the cytosolic monothiol glutaredoxin-3 (GLRX3) are reductively coupled to form a $[4Fe\text{-}4S]^{2+}$ cluster, thanks to the electrons donated *in vitro* by glutathione (GSH) [4].
Here, with the aim of further contributing to the molecular understanding of the mechanism of [4Fe-4S] proteins biogenesis in the cytosol, we investigated the role of human anamorsin, which is a partner protein of GLRX3 [5] acting as an electron transfer protein in cytosol [6-8], as the possible physiological electron donor in the formation of the $[4Fe\text{-}4S]^{2+}$ cluster at the N-terminal site of NUBP1. We found that a hetero-tetrameric complex formed by one molecule of dimeric cluster-oxidized $[2Fe\text{-}2S]^{2+}{}_2$-$GLRX3_2$ and two molecules of cluster-reduced $[2Fe\text{-}2S]^{+}{}_2$-anamorsin performs the assembly of the N-terminal $[4Fe\text{-}4S]^{2+}$ cluster of the cytosolic protein NUBP1.

P13. STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF PROTEINS INVOLVED IN IRON-SULFUR CLUSTERS BIOGENESIS IN *MYCOBACTERIUM TUBERCULOSIS*: TOWARDS THE DISCOVERY OF NEW ANTIBACTERIAL DRUGS?

Ingie Elchennawi, Christelle Caux, Philippe Carpentier, Marine Ponge and Sandrine Ollagnier de Choudens

Univ. Grenoble Alpes, CNRS, CEA,
Laboratoire de Chimie et Biologie des Métaux,
F-38000 Grenoble, France

Ingie.elchennawi@cea.fr

Iron-sulfur (Fe-S) clusters are inorganic cofactors that are essential in various biological processes (e.g., DNA repair, respiration, photosynthesis, cofactor biosynthesis) [1]. They exist in different forms and oxidation states, the most common types being [4Fe-4S] and [2Fe-2S] clusters. In bacteria, two major pathways for Fe-S cluster biogenesis and delivery have been identified: the Isc and the Suf systems. In *Escherichia coli*, the Isc system is the housekeeping pathway for Fe-S biogenesis, whereas the Suf system is active under environmental stress and iron limitation [2]. Interestingly, Mycobacterium *tuberculosis* (*Mtb*), the causative agent of Tuberculosis (TB), contains only the Suf system as Fe-S assembly machinery [3]. Suf system is essential for *in vitro* growth of *Mtb* under normal conditions [4,5] and recently proved to be a point of vulnerability in *Mtb* [6]Moreover, the Suf components of *Mtb* are induced during iron starvation, a process experienced by the pathogen in host tissues, indicating that Fe-S assembly and therefore Fe-S metabolism may be important in the establishment of latent infection [7]. Similarly, the *Mtb* Suf system is up-regulated under nitrosative and oxidative conditions, stressors of the innate immune response [8]. Therefore, there is mounting evidence that Suf sytem is essential for *Mtb* pathogenicity and targeting it might open novel avenues for the development of novel anti-TB drugs through disturbing the pathogen's Fe-S metabolism. A requisite of that consists in characterizing *Mtb* Suf proteins.
Structural and functional characterization of the Suf proteins from the *Mtb* suf operon is mostly unknown and constitutes the main goal of our research. We will present the first characterization of two proteins of the Suf system from *Mtb*.

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P14. STUDY OF FERRITIN AND FERRITIN DERIVATIVES IN THE PATHOLOGY OF NEURODEGENERATIVE PROCESSES

Ján Gomboš[1], Lucia Balejčíková[2], Marianna Baťková[3], Peter Kopčanský[3], Oliver Štrbák[4]

[1] Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia; [2] Department of Hydrology, Slovak Academy of Sciences, Bratislava, Slovakia; [3] Institute of Experimental Physics, Slovak Academy of Sciences, Košice, Slovakia; [4] Biomedical Centre Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia.

gombos8@uniba.sk

Iron belongs to the group of essential elements in our body. It is involved in many fundamental metabolic processes. Iron intake, usage, and storage are strictly regulated and must work flawlessly since a very thin line between the positive and negative effects of iron exists. Disruption of iron homeostasis can lead to various pathological processes associated with decreased iron-sulfur cluster production (ISC) and excessive iron accumulation in tissue. Ferritin has been proposed as a precursor of iron accumulation. Ferritin stores biologically non-toxic Fe^{3+} form in its mineral core. Due to unknown mechanisms, the core can change its composition to mixed Fe^{3+} and Fe^{2+} form, forming so-called pathological ferritin with a magnetite-like mineral core. It is unclear whether magnetite in so-called pathological ferritin is a trigger or consequence of pathological processes associated with neurodegenerative diseases, such as Alzheimer's disease. To simulate pathological ferritin, we use magnetoferritin, which consists of apoferritin and artificially added magnetite. Magnetoferritin can help determine whether iron accumulation is a trigger or a consequence of neurodegeneration. In addition, it can help to reveal the fate of the "lost" iron from ISC synthesis during neurodegeneration. We studied the viability of the model neuronal system of cells in the presence of ferritin and magnetoferritin, as well as different iron-reducing agents (ascorbic acid, riboflavin, lysozyme amyloid fibrils). We found an increased release of potentially toxic Fe^{2+} ions from the mineral core of pathological ferritin compared to native ferritin. This can help us understand the toxicity of accumulated iron and its potential to induce neurodegenerative processes.

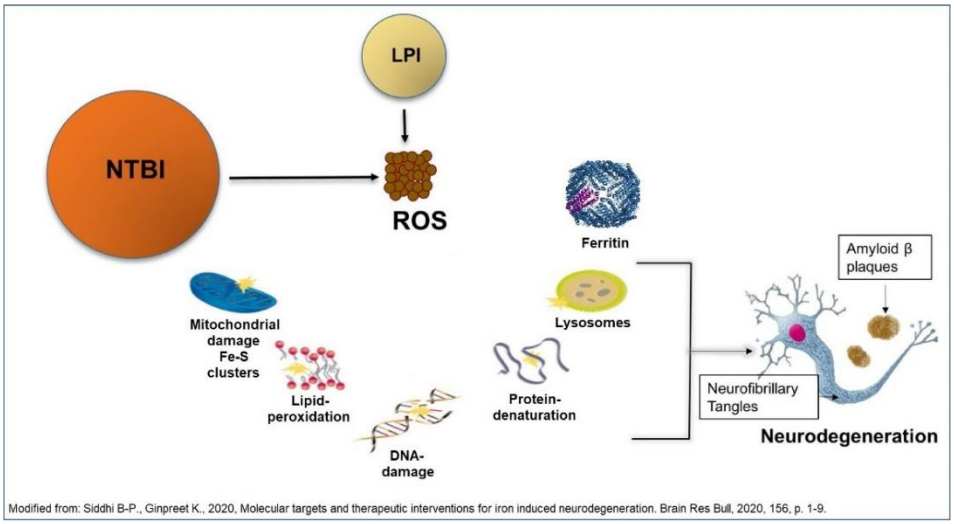

Figure 1. Intracellular damage caused by Reactive oxygen species. NTBI: Non-transferrin-bound serum iron; LPI: Labile plasma iron; ROS: Reactive oxygen species.

P15. BIOINORGANIC IMMUNOLOGY AND DRUG DISCOVERY; FROM METALLOPROTEINS FUNCTION TO DRUG DISCOVERY

Kourosh H. Ebrahimi, Astrid Maluta, Hoang Thao Nghi, Yujie Sheng, Mohammed Hakil,

Institute of Pharmaceutical Science,
King's College London, London, UK

kourosh.ebrahimi@kcl.ac.uk

Our overarching objective is to understand the roles of metals and metalloproteins, including iron-sulfur enzymes, in the innate immune response and cancer. We use the outcomes of our work to discover and develop new therapeutics. The current research in the group focuses on three areas:

1. We have discovered a fungal radical S-adenosylmethionine (SAM)-dependent nucleotide dehydratase (SAND) [1]. We showed that the enzyme produces antiviral nucleotide analogues [2]. These molecules are one of the largest groups of FDA-approved antiviral drugs. We aim to engineer the enzyme and discover its homologues to synthesise antiviral nucleotide analogue therapeutics using environmentally friendly enzymatic/chemoenzymatic processes.

2. Ferritin nanocage is the ubiquitous iron-storage protein of life [3]. There is a growing interest in using this nanocage for drug delivery and creating vaccines [4]. We are developing a new ferritin nanocage technology to encapsulate various drugs under benign conditions. Our technology will enable us to furnish the surface of ferritin nanocages with multiple ligands and generate mosaic therapeutics like vaccines.

3. We have shown that iron-sulfur proteins play vital roles in various aspects of the innate immune response [5]. Our data suggest that the human antiviral enzyme SAND modulates immunometabolism [6]. We are investigating the role of iron-sulfur proteins, namely human SAND, mitoNEET and Elp3, in the immune response and cancer. We aim to understand how these proteins function and modulate oncogenes (Figure).

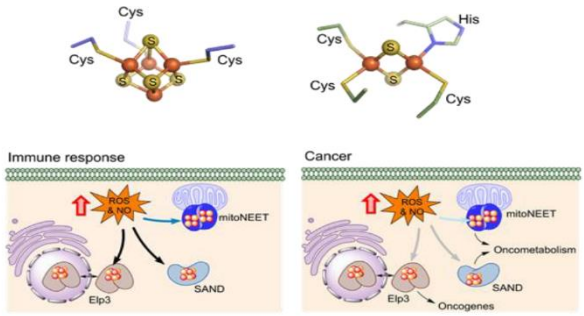

Figure. Two forms of iron-sulfur [FeS] clusters present in Elp3, mitoNEET and SAND. We propose that the clusters act as a "biological fuse" or sensor. These roles of the clusters during the innate immune response provide a feedback mechanism to prevent excessive response. In cancer cells, the functions of the clusters are disrupted, and the activity of enzymes activates oncogenes and modulates oncometabolism.

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P16. MODULATION OF IRON METABOLISM IN MITOCHONDRIA AS A NEW CHEMOTHERAPEUTIC STRATEGY

Lukáš Werner[1], Robert Sutak[2], Dominik Arbon[2], Jan Stursa[1], Cristian Adrian Sandoval Acuna[1], Jaroslav Truksa[1]

[1] Institute of Biotechnology, Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic; [2] Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic

sutak@natur.cuni.cz ; lukas.Werner@ibt.cas.cz

Rapidly dividing cells, such as cancer cells or parasitic microorganisms, have higher iron demand to fulfill their metabolic needs and enhanced biosynthesis of cellular components that are necessary for sustaining cellular proliferation. Iron is a critical cofactor of many metabolic enzymes, mostly in form of iron-sulfur clusters and heme, both of which are synthesized in mitochondria. Thus, interfering with mitochondrial iron metabolism affects cellular ability to proliferate. Herein we present that inhibition of mitochondrial iron metabolism has effect against cancer cell lines (4T1, MCF7, MDA-MB-231) as well as against eukaryotic parasitic microorganisms such as the causative agent of sleeping sickness *Trypanosoma brucei* or the pathogenic amoeba *Acanthamoeba castellanii*.

The newly synthetized derivatives of deferoxamine (mitoDFO) with various mitochondrial vectors take advantage of the distinct functioning of cancer/pathogen mitochondria and preferentially affects cancer/parasitic cells while sparing non-malignant/host cells depending on vector structure. Furthermore, the tested compounds do not alter systemic iron metabolism in preclinical mouse models, corroborating the preferential targeting of cancer/pathogenic cells.

At the molecular level, in the cancer cells the compounds interfere with the mitochondrial biosynthesis of iron-sulfur clusters and heme, leading to inhibition of mitochondrial respiration, disassembly of mitochondrial respiratory super-complexes and generation of reactive oxygen species. In brief, it causes profound mitochondrial dysfunction and induces mitophagy. In the model parasitic protist *Trypanosoma brucei*, a significand part of the mode of action is modulation of the physicochemical properties of the inner mitochondrial membrane.

P17. CIGARETTE SMOKE ALTERS MACROPHAGE MITOCHONDRIAL IRON HANDLING IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: IMPLICATIONS FOR HOST-PATHOGEN INTERACTIONS

Faherty L [1, 2], Kim K [3], Zhang WZ[3], O'Beirne SL [3], Salit J [3], Rostmai MR [3], Kaner RJ [3], Crystal RG[3], Healy C [1, 2], Cloonan SM [1, 2, 3]

[1] School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; [2] Tallaght University Hospital, Dublin, Ireland; [3]Joan and Sandford I. Weill Department of Medicine, Division of Pulmonary and Critical Care Medicine, Weill Cornell Medicine, New York City, USA

fahertyl@tcd.ie

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease characterized by emphysema and chronic bronchitis [1]. Cigarette smoke (CS) inhalation constitutes the primary risk factor for COPD progression and dysregulates numerous pathways essential to lung-resident alveolar macrophage (AM) and circulating bone marrow-derived macrophage (BMDM) function [2]. Aberrant iron metabolism is one such hallmark: AMs are the primary lung resident iron-positive cells, with numerous iron-related proteins elevated in patient bronchoalveolar lavage fluid [3, 4]. Interestingly, CS also causes mitochondrial dysfunction in macrophages, which are the primary iron storage sites in the cell and are responsible for the production of iron-containing cofactors, including iron-sulfur clusters [5]. While CS is known to associate with mitochondrial dysfunction in COPD, its effect on mitochondrial iron in COPD macrophages, and the role of such in dictating the outcome of host-pathogen interactions, is unknown.

Interrogation of bulk RNA-seq data of AMs isolated from COPD patients and healthy smoker controls revealed significantly altered expression patterns of genes associated with mitochondrial iron metabolism, with the expression of such factors further investigated in BMDMs and AMs isolated from CS-exposed mice as an *in vivo* model of COPD. Using *Streptococcus pneumoniae*, one of the most commonly cultured pathogens from the COPD lung, we show that infection of an immortalised BMDM cell line (iBMDM) *in vitro* causes rapid intracellular total iron and heme loss as determined through graphite furnace atomic absorption spectrometry and heme assay, respectively. Macrophage mitochondrial iron loading through ferric ammonium citrate treatment further exacerbates this phenotype and associates with increased numbers of internalised bacteria, while iron depletion using the mitochondrial iron chelator deferiprone is protective against this bacterial-induced iron loss. These findings illustrate the role of macrophage mitochondrial iron dysregulation in COPD pathogenesis and present the manipulation of mitochondrial iron in the lung as an intriguing therapeutic target, both in COPD and in other diseases characterised by recurrent acute infections.

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P18. DIFFERENTIAL DIAGNOSIS OF ANEMIAS IN THE LABORATORY

Anila Mitre1[1, 2], Merita Rumano2[1]

[1] Tirana University, Faculty of Natural Sciences, Biology Department; [2] Medical laboratory-Intermedica Diagnostic Center

meritarumano@yahoo.com

Background:
The aim of this study is to identify and classify anemias based on laboratory analysis. Taking into consideration that anemias can be of pathophysiological or morphological origin, our objectives is linked to the evidence of anemias based on the hemogram parameters, such as Red Blood Cells (RBC), Hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and hematocrit (Hct); evidence of congenital hemoglobinopathies through hemoglobin electrophoresis; evidence of anemia related to the lack of vitamins B12 and Folic acid and the evidence of anemias related to the reduction of Iron deposits (low Ferritin level).
Methods:
2360 individuals aged from 1-97 years old were included in this study. RBC, Hb, Hct, MCV and MCH were measured in the Sysmex apparatus, while for the cases presenting low levels of Hb, MCV and MCH, Hb electrophoresis was done at Sebia apparatus. Vitamin B12, Folic Acid and Ferritin were measured using the chemiluminescent technique by Cobas 6000 Apparatus.
Results:
After completing blood analysis, it was found that only 1620 individuals present the condition of anemia. In total, only 1.28% of the individuals included in the study present hemoglobinopathies. Hb electrophoresis showed that only 1 individual was suffering from sickle cell disease and 2 other individuals from thalassemia. 28 cases were carriers of thalassemia and 11 others were carriers of sickle cell anemia.
As a result we can say that 30% of the cases result with mild anemia, 25% result in anemia because of infections, 9.3% due to the lack of Vitamin B12, 0.5% due to folic acid deficiency and 0.1% due to the lack of folic acid and vitamin B12 (lack of both). In 7.6% of cases, the absence is related to hemorrhagic reasons.
Conclusion:
Knowing the cause of anemia and classifying them according to blood analysis findings, will help not only to better treat this condition, but will also help to design appropriate policies and interventions for the prevention and control of anemia in Albania.

THE STRUCTURE OF A NOVEL FERREDOXIN – FHUF, A FERRIC-SIDEROPHORE REDUCTASE FROM *E. COLI* K-12 WITH A NOVEL 2FE-2S CLUSTER COORDINATION

Trindade I.B.[1], Rollo, F.[1], Todorovic, S.[1], Catarino, T.[1,2], Moe, E.[1], Matias, P.M.[1,3], Piccioli, M.[4], Louro, R.O.[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República, 2780-157, Oeiras, Portuga
[2] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal
[3] iBET – Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal
[4] Magnetic Resonance Center and Department of Chemistry, University of Florence, Via L. Sacconi 6 50019 Sesto Fiorentino, Italy

louro@itqb.unl.pt

Iron is a vital element for life. However, after the Great Oxidation Event (GOE), the bioavailability of this element became limited. To overcome iron shortage and to scavenge this essential nutrient, microorganisms use siderophores. These are secondary metabolites that have some of the highest affinities for ferric iron. Upon reuptake of the ferric form of siderophores by the cells, the iron needs to be released to be integrated into cellular components. This crucial step is mediated by Siderophore-Interacting Proteins (SIPs) or Ferric-siderophore reductases (FSRs).
In this work, we report for the first time the structure of an FSR. FhuF from laboratory strain *Escherichia coli* K-12 is the archetypical FSR, known for its atypical 2Fe-2S cluster with the binding motif C-C-X10-C-X2-C. The 1.9 Å resolution crystallographic structure of FhuF shows a novel coordination of the 2Fe-2S cluster that matches the unusual spectroscopic properties of FhuF. FhuF shows an impressive ability to reduce hydroxamate-type siderophores at very high rates when compared to flavin-based SIPs, but like SIPs appears to use the redox-Bohr effect to achieve catalytic efficiency.
Overall, this work closes the knowledge gap regarding the structural properties of ferric-siderophore reductases and simultaneously opens the door for further understanding of the diverse mechanistic abilities of these proteins in the siderophore recycling pathway.

P20. IRON-SULFUR CLUSTER SYNTHESIS AS A DRUG TARGET FOR HUMAN PARASITE *TOXOPLASMA GONDII*

Eléa A. Renaud, Sarah Pamukcu, Aude Cerutti, Laurence Berry, Yann Bordat, Sébastien Besteiro

LPHI, Univ Montpellier, CNRS, UMR5235, Montpellier, France

sebastien.besteiro@inserm.fr

Toxoplasma gondii is a unicellular eukaryotic parasite that is responsible for a disease called toxoplasmosis in humans and other vertebrates. While toxoplasmosis is mostly asymptomatic in immunocompetent individuals, it can be harmful for developing fetuses or for immunocompromised patients. *T. gondii* harbors a non-photosynthetic plastid, called the apicoplast, that was inherited from a secondary endosymbiosis. As a consequence, like in land plants and algae, this parasite has three different Iron-Sulfur cluster synthesis pathways localizing to the mitochondrion (ISC), the cytoplasm (CIA) and the apicoplast (SUF), respectively.

Our recent work has shown that disrupting the mitochondrial or the apicoplast-based pathways, while both important for parasite growth, leads to markedly different fates [1, 2]. Impairment of the mitochondrial pathway triggers parasite differentiation into a stress resistance stage, allowing survival to some extent. On the other hand, impairment of the SUF plastidic pathway leads to parasite death, likely due to perturbations in a number of downstream cellular functions, including the synthesis of isoprenoid precursors. This validates the SUF pathway as a potential drug target in *T. gondii*, because it is essential for parasite survival and it is absent from the mammalian host. Moreover, beyond the SUF machinery and even in Iron-Sulfur cluster synthesis pathways conserved in mammals like the CIA system, *T. gondii* also shows peculiarities that may be exploited for drug design. Finally, as *T. gondii* is an obligate intracellular parasite, it must obtain iron from host tissues and must regulate iron homeostasis to avoid toxicity, which may also offer opportunities for antiparasitic strategies.

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P21. UNVEILING THE ROLE OF TWO UNUSUAL ENDONUCLEASE III ENZYMES IN THE EXTREMOPHILE *DEINOCOCCUS RADIODURANS*

Shakiba Gholami, Filipe Rollo, Filipe Folgosa, Smilja Todorovic and Elin Moe

Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República, 2780-157, Oeiras, Portuga

shakiba.gholami@itqb.unl.pt

The bacterium *Deinococcus radiodurans* is well known for its exceptional radiation resistance and capacity to endure hostile environments. Although most bacteria have unique gene coding, *D. radiodurans* carries three genes encoding Endonuclease III: DR2438 (DrEndoIII1), DR0289 (DrEndoIII2), and DR0982 (DrEndoIII3). In each of these three enzymes, a DNA-binding cleft separates two α-helical domains, which contain a [4Fe-4S] cluster and a HhH-motif [1]. The role of the cluster is still poorly understood and controversial. We have been studying the three enzymes in parallel, at different levels, in order to unravel their particularities. Based on biochemical analysis, DrEndoIII2 seems to be the main enzyme in this organism, while the the roles of DrEndoIII1 and DrEndoIII3 are not fully understood [2]. In particular, the function of DrEndoIII3 is a mystery since it does not show any activity on classical EndoIII substrates under standard assay conditions. It has previously been shown that a hypothetical uracil DNA glycosylases in *D. radiodurans* are active only at low pH [3]. Here we have performed activity assays of DrEndoIII3 at pH ranging from pH 5 – 9 in order to investigate the pH dependence profile of this enzyme. Biochemical studies indicated that DrEndoIII1 acts mainly as a glycosylase and also possesses specificity for single stranded damages [2]. Here we report results from EPR experiments of DrEndoIII1 in presence and absence of DNA under native and oxidizing conditions.

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P22. WHAT CAN NANOFORMULATIONS DO TO SOLVE DELIVERY PROBLEMS OF PIS: EXAMPLES WITH LIPOSOMES

Sophia G. Antimisiaris[1, 2]

[1] *University of Patras, Department of Pharmacy, Laboratory of Pharmaceutical Technology, Patras, Greece*
[2] *FORTH/ICE-HT, Institute of Chemical Engineering Sciences, Laboratory of Pharmaceutical Technology, Patras, Greece*

santimis@upatras.gr

The application of nanotechnology in Pharmacy and especially in the development of sophisticated formulations for drug administration and/or targeted delivery, has led to the development of therapeutic systems or nanotherapeutics with exceptional advantages. The most recent and well-known development in the area is the successful development of mRNA vaccines against the virus that causes SARS-COV-2 (severe acute respiratory syndrome coronavirus 2).

In general, the development of advanced drug delivery systems has led to systems with exceptional advantages in terms of the therapeutic effect achieved. Many of the problems for administration of drugs, that are related with either the limited stability of drugs and/or their physicochemical properties (high molecular weight, low permeability through biological barriers, low solubility), can be solved by innovative nanomedicines. In this presentation, specific examples of ways to overcome drug administration problems from our recent research projects, exploiting also alternative or topical route of drug administration, will be presented.

Focus will be on liposome applications in the areas of: (i) Localized delivery of liposomal drugs with examples on intrapleural and ocular delivery [1-3], (ii) Potential therapeutic advantages of liposomal antimicrobials [4,5]; (iii) Targeted liposomes, as nanoparticle based drug delivery systems for brain-located pathologies, such as Alzheimer's Disease (AD) [6-9]. (iv) Other applications [10, 11].

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23. THE LONDON METALLOMCS FACILITY

Wolfgang Maret[1], Alexander Griffiths[2], Alexander P. Morrell[2]

[1] Department of Nutritional Sciences, School of Life Course and Population Sciences, Faculty of Life Sciences and Medicine, King's College London; [2] The London Metallomics Facility, Research Management and Innovation Directorate, King's College London

wolfgang.maret@kcl.ac.uk

The London Metallomics Facility (LMF) is a core research facility at King's College London and supported by many groups in London with their research activities in metallomics - global approaches to metals in the biosciences. It developed from a Wellcome Trust grant (2016-2021) that included researchers from King's College and Imperial College London [1]. The grant allowed the acquisition of instrumentation for laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for metal imaging and for total X-ray reflection fluorescence measurement (TXRF), with one goal to start developing a human metal atlas. King's College London, with the support of commercial partners, furthered the development of the LMF, becoming one of the college's fastest growing core facilities. The LMF now contains a designated clean sample area for work under metal-controlled conditions and equipment for complex digestions. The facility now has an ICP-QQQ-MS, the latest technology for laser ablation, and, within the next months, will have an ICP-TOF-MS, both designed for fast throughput and detection of nearly the entire periodic table simultaneously. Integration into King's core facilities provided further opportunities for collaborations with other centres at King's for correlative bioimaging that includes many types of microscopies, spectroscopies, and molecular mass spectrometries for multi-modal investigations of the roles of chemical elements in health and disease [2]. The London Metallomics Consortium (LMC), which includes several universities in London, supports the efforts of the LMF with additional activities such as symposia, educational events, and developing engagement and funding strategies. All these developments give the field of metallomics an impetus to grow with new applications and workflows that include other omics approaches in the biosciences such as genomics, proteomics, lipidomics [3]. LMF interfaces with other research groups employing oxygen ion plasma secondary ion mass spectrometry (SIMS) and multicollector (MC)-ICP-MS (both at Imperial College), positron emission tomography (PET) (at King's), and facilities at the Rosalind Franklin Institute. The LMF is part of Euro-BioImaging ERIC (European Research Infrastructure Consortium). Notably, we are a service facility that considers projects from the UK, Europe, and other continents. The LMF would be delighted to discuss any of your project needs and encourages you to reach out with any questions. **London Metallomics Facility (kcl.ac.uk)**

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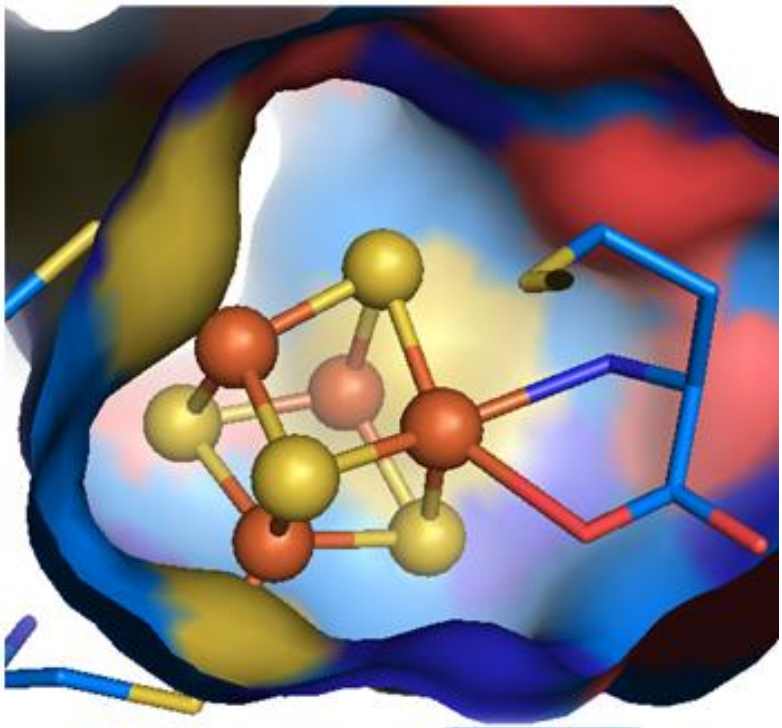

COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.