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7th National Meeting of Portuguese Synchrotron Radiation Users

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WELCOME TO THE 7th ENURS 2018

For this year of 2018, the organization of the National Meeting of Portuguese Users of Synchrotron Radiation (ENURS) became the responsibility of the Materials Science Department of the Faculty of Sciences and Technology of NOVA University of Lisbon.

It is the 7th Edition of this important gathering of a very special community that became interested in the use of Synchrotron Radiation for the development of new frontiers in science and technology.

The ENURS tradition says that all are welcome to participate without registration fees, creating a special atmosphere of closeness and friendship, allowing for a wide scope interaction and networking between participants. There was a time when only a few used SR for their work. That is now changed, with the ever-increasing community profiting from the expanding available facilities. This is patent in the number of selected oral communications and poster contributions.

ENURS has a special support from international organizations that invest in the dissemination of knowledge fostered in their facilities and in opportunities and incentives for researchers. This year we are happy to have with us three reputed institutions. Representatives from ALBA (Barcelona, Spain), BESSY II (Berlin, Germany) and ESRF (Grenoble, France) will speak about the research produced in their facilities, beamlines available and access incentives to their infrastructures.

The foundations were established, and the consolidation of the various research fields, with the enthusiasm of new users and encouragement of more experienced ones, makes this informal meeting the perfect stage to establish bridges, not only among our national community of SR users, but also with the invited international experts.

It is a pleasure and an honor to be part of this.

João Pedro Veiga

Monte de Caparica, 8th of June 2018

HORIZON 2020
GA 700395

AGENDA

June, 8th

ENURS 2018

Venue: Biblioteca | FCT @ Campus Caparica

09:30 – 10:00	**Registration, Welcome Coffee and Poster Set-Up**	
10:00 – 10:15	Welcome Session	João Pedro Veiga
10:15 – 10:30	Information from the National Delegate at the ESRF	Maria João Romão
10:30 – 10:35	Information from the ESUO Portuguese Delegate	F.M. Braz Fernandes
10:35 – 11:05	Research at ALBA	Salvador Ferrer
11:05 – 11:35	Research at BESSY II – your soft X-ray light source in Europe	Ana Sofia F. Anselmo
11:35 – 12:05	Research at ESRF	Harald Reichert
12:05 –14:00	**Lunch and Poster Session**	
14:00 – 14:30	**Invited Speaker 1** - Metal distribution mapped by X-ray fluorescence nano-imaging in Deinococcus radiodurans and Saccharomyces cerevisiae	Célia Romão ITQB NOVA
14:30 – 15:00	**Invited Speaker 2** - In situ studies of materials processing.	F.M. Braz Fernandes FCT NOVA
15:00 – 15:15	**O1** - Following the octahedra distortions of $RFeO_3$ and $RMnO_3$ by XRD and EXAFS at high-pressure	Rui Vilarinho Silva FC UPorto
15:15 – 15:30	**O2** - The use of time-resolved small-angle x-ray scattering techniques to evaluate the molecular organisation changes in 3d printed TPU	Saba Abdulghani Oliveira da Silva IPLeiria
15:30 – 16:00	**Coffee Break and Poster Session**	
16:00 – 16:15	**O3** - High resolution X-ray powder diffraction for small drug molecules structure solution	Inês Martins IST UL
16:15 – 16:30	**O4** – Crystal structure of Human p53 R280K mutant (DNA binding domain) – Explaining the loss of DNA binding	Filipa Trovão FCT NOVA
16:30 – 16:45	**O5** - Effects of laser processing on the transformation characteristics of NiTi: A contribute to additive manufacturing using synchrotron X-ray diffraction	João Oliveira FCT NOVA
16:45 – 17:00	**O6** - Structural and functional studies on MsmX, a model for multitask ATPases in bacteria	Francisco Leisico FCT NOVA
17:00 – 17:15	**Best Poster Prize and Closing Remarks**	

Scientific Communications

Invited Speakers

Metal distribution mapped by X-ray fluorescence nano-imaging in *Deinococcus radiodurans* and *Saccharomyces cerevisiae*

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Keywords: *Bacteria*, yeast, X-ray fluorescence microscopy, oxidative stress, manganese

Metals play essential and crucial roles in the most important and chemically challeging processes in life. Therefore the intracellular localization, speciation and distribution of metals is of great interest. Using X-ray fluorescence imaging, we have been addressing element/metal localization and distribution in the radiation resistant bacterium, *Deinococcus radiodurans,* and in yeast, *Saccharomyces cerevisiae*.

D.radiodurans is the most radiation resistant organism known to date, moreover it is also highly resistant to other conditions, such as desication or oxidative stress conditions [1]. The proposed mechanism that avoids cell death relies on the protein protection against oxidation [2], promoted by the oxidative stress generated under these extreme conditions. Besides the canonical enzymatic systems, such as superoxide dismutase and catalase, this bacterium contains a highly efficient non-enzymatic system that involves small complexes of manganese and other small molecules, such as phosphate [3]. We have used synchrotron X-ray fluorescence nano-imaging at ID16A beamline - ESRF in order to localize manganese and phosphate in *D.radiodurans* in control conditions and to understand their distribution under stress conditions. Our results show that cell protection against ROS is dependent on the metal distribution inside the cell.

Over the last years integrated biophysical and biochemical approaches have demonstrated that vacuoles (acidic organelles) and mitochondria are the main stores of iron in single-celled eukaryote *S. cerevisiae* [4]. In the yeast *Saccharomyces cerevisiae*, iron accumulation in vacuoles is mediated by the vacuolar transporter Ccc1, which is crucial for yeast survival under Fe-loading conditions. Accordingly, it was shown that deletion of the gene encoding the vacuolar Fe importer Ccc1 is lethal under iron-loading growth conditions and leads to mitochondrial iron accumulation [5]. Synchrotron X-ray fluorescence imaging conjugated with organelle fluorescence microscopy [6] or with light microscopy [7] emerges as the most suitable and unbiased approach to study the yeast intracellular *ironomics,* as permits to map Fe distribution in the whole cell. We have acquired X-ray fluorescence imaging data at ID16B nano-analysis beamline, in order to better understand the mechanism of iron homeostasis and its dependence on Ccc1.

In conclusion, the X-ray imaging data presented in this study (ID16A and ID16B) have proved to be essential to elucidate element/metal homeostasis in both model organisms, *Deinococcus radiodurans and Saccharomyces cerevisiae*.

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In situ studies of materials processing

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Keywords: In situ XRD, thermomechanical processing, composites, shape memory alloys, functionally graded materials.

In situ studies of fabrication processes of metallic alloys were carried on at ESRF (BM-20) and DESY (HEMS-P07) covering the following processes: metal matrix composites casting, thin film sputtering, thermomechanical processing, welding and functionally graded materials. Main achievements in the following topics are illustrated in the current presentation:

- evaluation of wetting characteristics in FGMMC [1],
- stacking sequence of thin film formation as a function of deposition parameters and substrate [2,3],
- cold / hot working and subsequent recrystallization processes [4,5],
- in service behaviour of endodontic files during rotation / flexion [6],
- welding of shape memory alloys [7],
- localized heat treatments for functionally graded wires / strips [8].

These examples cover applications in automotive industry, MEMs, dentistery (ortho- and endodontics), civil engineering, aeronautics.

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Oral Communications

Following the octahedra distortions of *R*FeO_3 and *R*MnO_3 by XRD and EXAFS at high-pressure

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Keywords: rare-earth oxides, high-pressure, Jahn-Teller distortion

The structural, electric and magnetic properties of rare-earth perovskites (*R*BO_3, *R* a rare-earth cation and B trivalent cation) are closely correlated to the distortions of the BO_6 octahedra underlying the P*nma* symmetry. It has been demonstrated that the physical behaviour of these materials can be rather well understood based on two main distortions: BO_6 octahedra rotations and cooperative Jahn-Teller distortion (CJTD), when B is a Jahn-Teller active ion. Their physics has been studied under hydrostatic pressure, where a pressure-driven insulator to metal transition (IMT) has been found. However, for B = Mn^{3+}, where CJTD is present, controversial studies have been carried out in R = La, where the CJTD deformation was firstly proposed to disappear on the insulating phase at 18 GPa, based on X-ray diffraction measurements (XRD) [1]. However, it has been later concluded from Raman spectroscopy and X-ray absorption spectroscopy (XAS) studies, that the CJTD persisted both at the insulating and metallic phases in $LaMnO_3$ [2].

Our work reports on the pressure-dependence of the octahedra distortions of *R*FeO_3 studied by XRD at the ESRF. As in this system the only octahedra distortion is their tilting, a deeper knowledge of how the tilts accommodate the pressure as a function of R-cation is obtained. Then, comparing the *R*FeO_3 results with the ones for *R*MnO_3, obtained by similar studies at the ESRF, we were able to single out the role CJTD plays as pressure is applied [3]. Recently, the CTJD was studied in detail on $GdMnO_3$ and $DyMnO_3$ by means of the first high-pressure XAS studies done at the ESRF. The pressure dependence of the Mn K-edge EXAFS of these compounds (up to 30 GPa) revealed an unexpected result: the Jahn-Teller distortion is strongly reduced up to 5 GPa, evidencing the existence of a non-reported low-pressure isostructural transition. This result will be compared with the $LaMnO_3$ case, and discussed from the symmetry and compressibilities points of view, along with new DFT calculations, in order to unravel the actual role of the Jahn-Teller distortion on the structure of *R*MnO_3 series.

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The use of time-resolved small-angle x-ray scattering techniques to evaluate the molecular organisation changes in 3d printed TPU

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Keywords: 3D printing, 4D structures, SAXS, Thermoplastic polyurethane

4D printing is defined as 3D printing with the time being the 4th dimension, where parameters such as shape, property, or functionality of a 3D printed structure can change as a function of time in response to an external stimulus such as heat, light or moisture. In this context, materials such as thermoplastic polyurethane (TPB), can be used . TPU is a versatile polymer that is soft and processable when heated, hard when cooled and capable of being reprocessed multiple times without losing structural integrity. TPU is renowned for many things including its high elongation and tensile strength; elasticity and its ability to resist, solvents, chemicals and abrasion. TPU belongs to the family of materials known as thermoplastic elastomers (TPE). Chemically, TPU is composed of block copolymer molecules with alternating rigid and flexible segments (Fig. 1). It is this combination that gives this material its elastomeric nature and makes it an ideal candidate for 4D printing. In order to fully understand all aspects of 3d printing we have performed Small-angle X-ray Scattering experiments using the ALBA NCD-Sweet beam line on parts prepared in this manner in order to follow the structural development as a function of time. We describe the experiments to be performed later this year which will test the efficacy of this approach and we discuss the type of information we hope to extract. We will use the results for further improve our experimental approach and understanding of this material for the purpose of 4D printing and subsequent application areas.

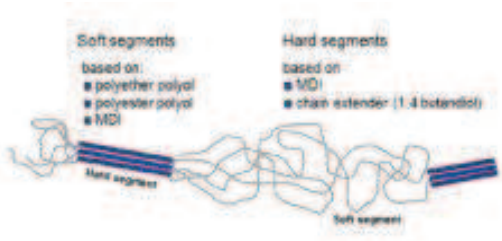

Figure 1 TPU structure

This work was supported by the Portuguese Foundation for Science and Technology (FCT) through the Projects references UID/Multi/04044/2013 and UC4EP (Ref. PTDC/CTM-POL/7133/2014). It was also funded by PAMI (ROTEIRO/0328/2013; Nº 022158), a Research Infrastructure of the National Roadmap of Research Infrastructures of Strategic Relevance for 2014-2020, co-funded by the FCT and European Union through the Centro2020. The small-angle x-ray scattering mesaurements were mad on the NCD-Sweet Beaamline at the ALBA synchrotron facility in Barcelona with the collaboration of ALBA staff and CALIPSOplus (Grant 730872) funding.

High resolution X-ray powder diffraction for small drug molecules structure solution

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Keywords: XRPD, SSNMR, DFT calculations, APIs, salts

As the vast majority of active pharmaceutical ingredients (APIs) occur as solids (~98%), solid-state structure-activity relationships are particularly relevant in pharmaceutical industry. For the characterization of small drug molecules, and their multicomponent crystal forms (co-crystals, salts, solid ionic liquids), X-ray diffraction (XRD) techniques (both single crystal and powder) are applied and entail detailed description of the intra- and intermolecular distances and angles. However, for probing local interactions involving light atoms, solid-state NMR (SSNMR) technique, highly sensitive to the local environment of a given nucleus, is often used in tandem with XRD data and *ab initio* density functional theory (DFT) calculations.[1] This combination of techniques (NMR crystallography) has been particularly useful for solving problems regarding the pharmaceutical co-crystal/salt frontier.[2]

Herein we report the successful results in the structure solution of adamantylamine (ADA) and folic acid (FA) multicomponent forms. XRPD data of FA samples were collected at the Swiss Light Source (SLS) synchrotron (PSI, Villigen, Switzerland) in the X04SA-MS beamline. Data collection at European Synchrotron Radiation Facility (ESRF), beamline ID22, on new FA multicomponent solid forms are schedule for July.

The authors acknowledge funding of PhD grant SFRH/BD/93140/2013 and project PEST-OE/QUI/UI0100/2013 by Fundação para a Ciência e Tecnologica. The authors also acknowledge SLS for granting the beamtime for the measurement through the Mesquick program and Dr. Nicola Casati for collecting the data.

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Crystal structure of Human p53 R280K mutant (DNA binding domain) – Explaining the loss of DNA binding

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Keywords: mutant p53R280K; crystal structure; DNA binding; anticancer therapy

The p53 tumor suppressor protein regulates cell proliferation, DNA repair, differentiation and death. This protein is widely found mutated in human cancer. The loss of p53 transcriptional activity may lead to tumor development and maintenance (1,2). For these reasons, p53 is seen as a promising target for therapeutic strategies to halt cancer. The crystal structure of mutant p53 R280K DNA binding domain (DBD) has been determined in the absence of DNA. X-ray diffraction data set from crystals of p53 R280K DBD were collected at the ID30A-3 beamline of the European Synchrotron Radiation Facility (Grenoble, France), with a resolution of 2.0 Å. The crystals belong to space group $P2_1$, with unit cell parameters a = 68.6, b = 69.4, c = 83.3 Å, β = 90.04º. The final model was refined to a final R factor of 19.4% (R_{free} = 23.7%) and contains four molecules of p53 R280K DBD in the asymmetric unit, four zinc ions and 339 water molecules. This structure was compared with the wild-type p53 core domain structures, alone and in complex with DNA. These comparisons contributed to a deeper understanding of mutant p53R280K structure. The increased distance and weaker binding of lysine 280 (mutation site) to DNA disables the formation of stabilizing interactions with DNA, explaining p53 R280K loss of binding (3). The structural information resultant from this study may also lead to further rational design of new potential anticancer therapeutic approaches, such as p53 reactivation molecules.

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Effects of laser processing on the transformation characteristics of NiTi: A contribute to additive manufacturing using synchrotron X-ray diffraction

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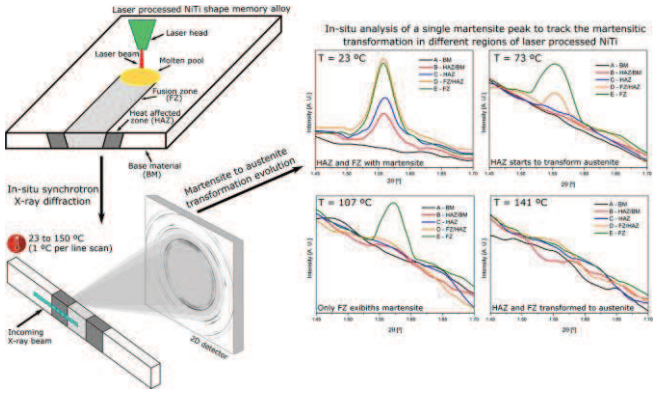
Keywords: NiTi, shape memory alloys, laser welding, additive manufacturing, synchrotron X-ray diffraction.

Additive manufacturing technologies allow to create complex shaped structures in a layer by layer fashion. NiTi shape memory alloys are now being extensively produced by laser-based additive manufacturing. The complex thermal history experienced during additive manufacturing can induced heterogenous precipitation phenomena. So far, no information regarding the transformation temperatures in NiTi parts obtained by additive manufacturing is found in the literature. This is due to the fact that conventional characterization techniques for NiTi shape memory alloys do not have the necessary special resolution to analyse separately each layer of deposited material. Each deposited layer creates a heat affected zone (in the precedent layer) and the last deposited layer as a structure typical as a fusion zone in a laser welded joint.

To have a better understanding of the laser/material interaction effects in NiTi parts produced by additive manufacturing we have used a simplified approach. This encompassed the use of a laser heat source to create a heat affected and a fusion zone in a NiTi sheet. Then in-situ X-ray diffraction using synchrotron X-ray diffraction (at P07 beamline at DESY) was used to track the martensite to austenite transformation in the different regions of the laser processed material. It was observed that a gradient of transformation temperatures was observed from the base material towards the centre of the fusion zone. This is related to two distinct phenomena: in the heat affected zone, Ni_4Ti_3 precipitation promote Ni-depletion; in the fusion zone, preferential Ni evaporation also has the same effect. The Ni depletion in these regions promote an increase in the transformation temperature of the material, thus promoting the formation of a mixture of martensite and austenite at room temperature, while the non-affected base material was fully austenitic. These results justify the peculiar mechanical and microstructural features in NiTi parts created by additive manufacturing and put in evidence the need for solubilization heat treatments to homogenise the chemical composition of those parts.



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Structural and functional studies on MsmX, a model for multitask ATPases in bacteria

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Keywords: *Bacillus subtilis*, MsmX protein, ABC transporters, Pathogenic bacteria, X-ray Crystallography, Small-angle X-ray scattering

ABC transporters are one of the largest and most diverse superfamily in nature and it is widespread in all domains of life, being responsible for the primary transport of molecules against a chemical gradient driven by ATP hydrolysis. Thus, they are composed of different proteins specifically related to the substrate they transport, including the cytoplasmic ATPase domain, meaning that each transporter has one specific ATPase. However, there are different examples, such as *Bacillus subtilis*, which uses MsmX protein as a multitask ATPase, able to interact with several transporters responsible for the uptake of different oligosaccharides[1]. *B. subtilis* is a well-known model to study Gram-positive bacteria, specially the pathogenic ones like *Streptococcus*, *Staphylococcus* and *Clostridium*. Since, multitask ATPases are involved in carbohydrate uptake that mediates bacteria colonization and pathogenesis in the hosts[2,3], we aim to characterize the MsmX protein structural and functionally. In this work, we solved the first crystal structure of MsmX (dataset collected at ESRF, ID23) at 1.9Å and we could identify the most well-known ATPase motifs for ATP binding and reaction mechanism. Combining SAXS information (data collected at ESRF, BM29) and bioinformatic tools we assessed to potential structural determinants for the promiscuity of this protein towards different transporters, which are being functionally tested *in vivo* through mutagenesis approaches. Functional mutants of MsmX are being produced *in vitro* for further biochemical characterization. The structural, functional and *in vivo* insights into MsmX multitask ability derived from this multidisciplinary project (Fig. 1) will integrate future structure-based drug design efforts to develop new drugs to fight bacterial infections.

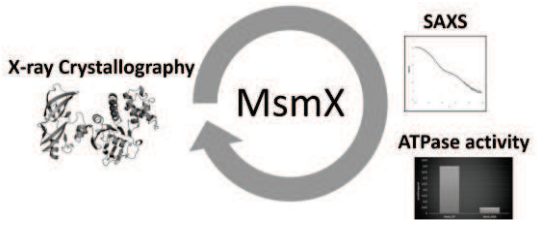

Figure 1. An integrative approach combining X-ray crystallography, SAXS experiments and functional studies was used to identify the molecular determinants involved in MsmX multitask ability.

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Poster Presentations

Analysis of a new flavodiiron protein structural arrangement in the enzyme from *Synechocystis* sp. PCC6803

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Keywords: Flavodiiron, oxidoreductase, cyanobacteria, class C FDP.

Flavodiiron proteins (FDPs) are involved in cellular response mechanisms against dioxygen and/or nitric oxide in prokaryotic and eukaryotic organisms [1]. FDPs minimal functional unit is composed by two structural domains, an N-terminal metallo-β-lactamase-like domain and a C-terminal flavodoxin-like domain. The first domain contains a diiron center, in which the reduction of O_2 (to H_2O) and/or NO (to N_2O) occurs, while the second domain has a FMN responsible for donation of electrons to the diiron site. Within each monomer, the distance between the FMN and the diiron center is too long (~ 40 Å) to allow a fast electron transfer between these two redox centers. Therefore, the "head-to-tail" dimerization in FDPs, brings the FMN from one subunit in close proximity to the diiron site of the other subunit, thus allowing an efficient electron transfer.

Based on the nature and sequence of additional C-terminal domains, FDPs have been classified into classes A-H [2]. The cyanobacterium *Synechocystis* sp. PCC6803 has four class C FDPs (Flv1-4) that contain an additional NAD(P)H:flavin oxidoreductase domain (Flv) at the C-terminal [3]. Two of them (Flv3 and Flv4) have the canonical residues involved in iron coordination, while the other two (Flv1 and Flv2) present non-canonical residues in this iron binding motif. Flv1 is classified as Class C-Type 2, the second most representative among the fifteen possible types [4].

Here, we report two crystal structures of a truncated Flv1 form, the as isolated form (Flv1_ΔFlv) and its iron soaked structure (Flv1_ΔFlv$_{Fe}$). Diffraction data were collected at beamlines ID30B and ID30A-3 of ESRF, for Flv1_ΔFlv and Flv1_ΔFlv$_{Fe}$, respectively. Both crystals belong to space group C2 and their structures were refined at 1.60 and 1.90 Å resolutions, respectively.

For the first time, the flavodiiron core of a class C FDP was determined. Details on the structural characterization on this FDP will be presented.

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Cloning, expression and purification of DNA polymerase A and NAD+-dependent DNA ligase from Deinococcus radiodurans

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Keywords: *Deinococcus radiodurans*, Base Excision Repair, DNA polymerase, DNA ligase

Deinococcus radiodurans is an extremophile bacterium with extreme resistance to desiccation and ionising radiation (rewieved in [1]). These extremophilic properties rely on an active proteome and efficient DNA repair systems [2]. One of the major DNA repair pathways is the Base Excision Repair (BER). DNA polymerase A family (*Dr*PolA) and NAD+-dependent DNA ligase (*Dr*LigA) from *D. radiodurans* are end processing BER enzymes, namely responsible for the correct base introduction and DNA strand sealing.

Here we describe the cloning, expression and purification of *Dr*LigA and *Dr*PolA, they were expressed in *E.coli* BL21 star pRARE2 and BL21 pLysS strain, respectively, and purified by affinity and gel-filtration chromatography. Crystallization screening experiments were performed in order to obtain crystals and to determine the protein structure by X-ray crystallography. The future aim is to perform structural and functional analysis of recombinant *Dr*LigA and *Dr*PolA for a better understanding of the molecular mechanisms leading to damaged bases repair. Moreover, as these enzymes are from an extremophile they may possess unique features and their potential in biomolecular applications should be explored.

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Tensile Loading of Functionally Graded Ni-Ti Shape Memory Alloy: In situ Structural Characterization

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Keywords: In situ XRD, Shape Memory Alloy, Functionally Graded Material

The present study focussed on the localized heat treatment (Joule heat effect, reaching 300ºC, 350 and 400ºC pulses for 10 minutes) of NiTi wires. In-situ synchrotron radiation-based x-ray diffraction has been used to study the tensile deformation. Initial scan before loading puts in evidence the presence of a graded microstructure along the localized heat treated zone (Fig. 1) Phase transformation sequence B2 – R – B19' during loading and reverse B19' – R- B2 during unloading is identified. This study will contribute to the development of graded heat treatment strategies that will profit at the most from the flexibility of the controlled heat treatment equipment developed at UNIDEMI.

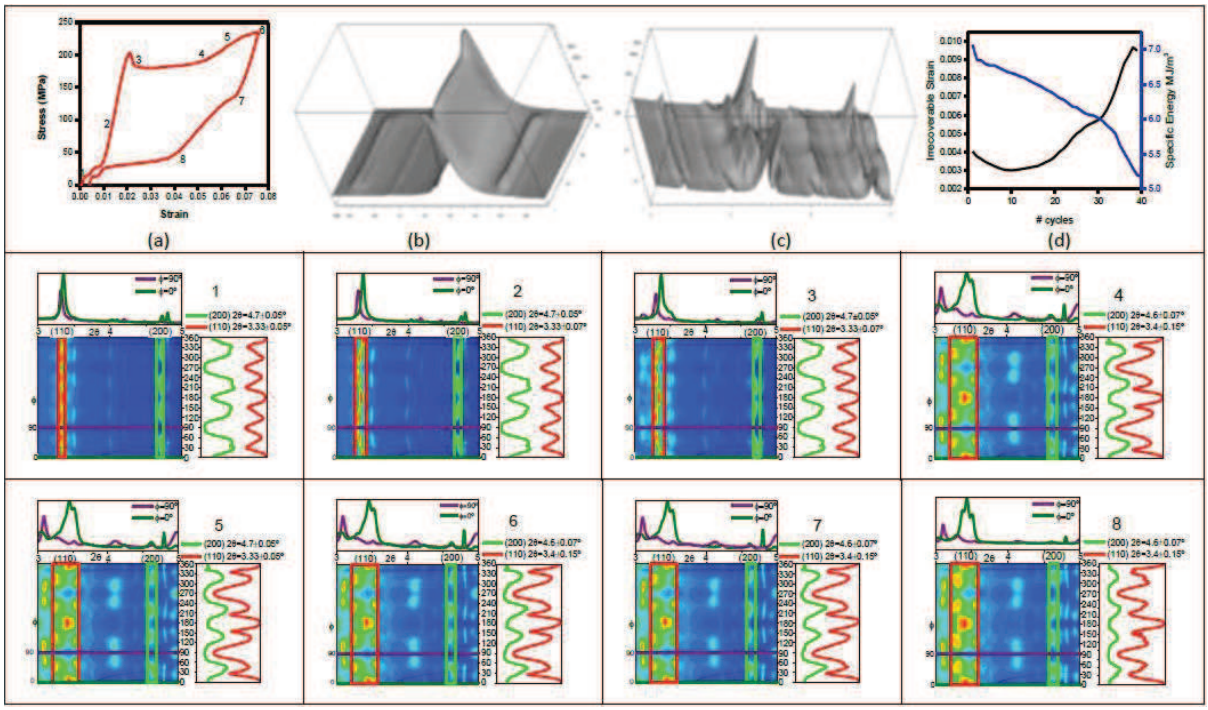

Fig. 1 - Localized heat treatment at 350ºC during 10 min: (a) one full superelastic cycle (load/unload); (b) 3D plot of the scan along the gauge length; (c) 3D plot of the structural evolution during one full superelastic cycle for the point at the center of the gauge length; (d) irrecoverable strain and specific energy absorbed along 40 successive superelastic cycles. At the 2nd and 3rd rows are represented the contour plots for 8 different points along the superelastic cycle (3°<2θ<5°; azimuthal angle between 0° and 360°); also plotted the 2θ scans for azimuths 0° and 90° (top of each contour plot) and maximum intensity for B2 (110) and B2 (200) versus azimuthal angle (at right of each contour plot).

Thermomechanical behaviour of shape memory rivet – In situ study

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Keywords: Ni-rich NiTi shape memory alloys, synchrotron radiation X-ray diffraction (SR-XRD), Ni_4Ti_3-precipitates.

In this study, various samples of a Ti-rich NiTi shape memory alloy (SMA) were used to join two components trough shape memory effect (SME). A recent patent [1] in the field of aeronautics opens interesting perspectives for this kind of joining processes. In the concept study and viability of a rivet with SME, DSC, dilatometric and in-situ XRD during thermomechanical cycles were performed [2]. In situ XRD study during thermomechanical cycle was conducted in a modified dilatometer DIL-805 (Bähr) at the HZG beamline (HEMS/P07-EH3, Petra III, DESY, Hamburg). The force exerted by SME is directly related to the initial texture of the material and the reorientation of the crystallographic variants. The maximum recovery reached by SME was 4.2%. The thermal hysteresis increases after the deformation of the samples. For the proof of concept, a sample of 5x5.5x5.5 mm3 was used, where pull-out tests were performed to simulate the separation of 2 sheets of metal, reaching up a maximum force of 340 N. Also, the actuation force by SME promoted a localized strain-hardening of the joined material, hence improving the contact interface between rivet and joined components.

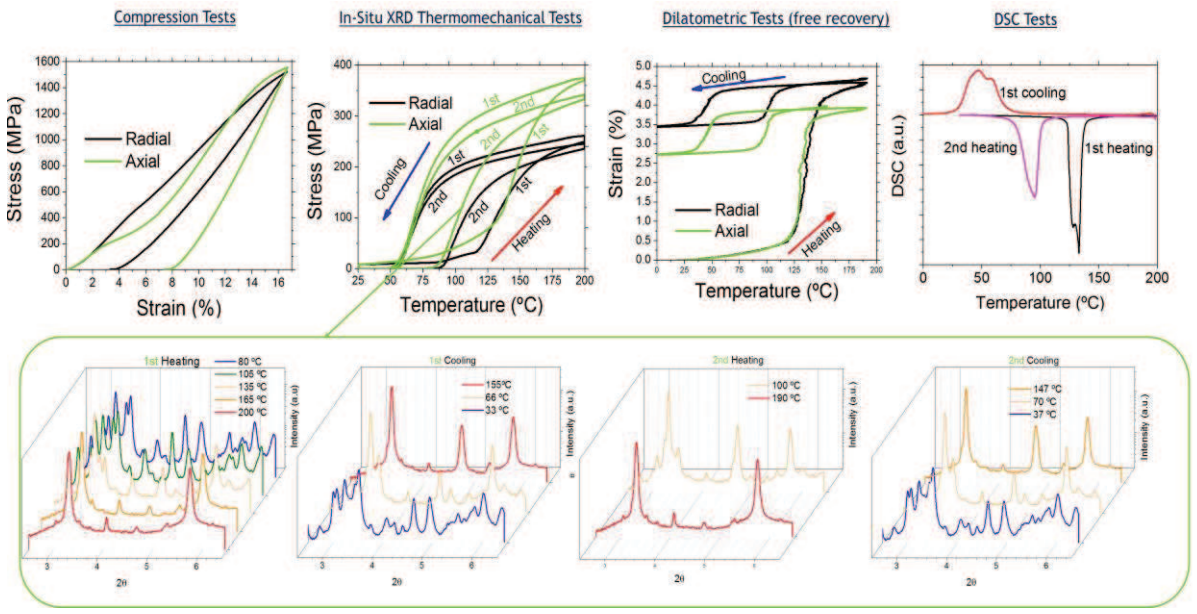

Fig. 1 – In situ study of the thermomechanical behaviour of Ti-rich NiTi shape memory alloy.

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MsmK, a decisive ATPase in *Streptococcus pneumoniae* virulence

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Keywords: *Streptoccocus pneumoniae*, MsmK, SAXS, *in vivo* assays

Streptococcus pneumoniae is an opportunistic human pathogen and the causal agent of many diseases, namely meningitis, otitis and pneumonia. Pneumonia is the deadliest infection in children under 5 years and kills more than AIDS, malaria, and measles together [1]. Sugars are the main carbon source for *S. pneumoniae*, thus ABC (ATP binding cassette) importers represent a crucial point in pneumonia progression. MsmK is an energizing multiple ABC importer responsible for carbohydrate uptake and deletion of this protein results in a lower colonization and attenuates the pathogen virulence (Fig.1) [2]. In this work, we combine in vivo and in vitro assays to characterize the activity of MsmK. We established the over expression of MsmK in *E. coli* cells and obtained stable and active protein at 30mg/mL. SAXS data was collected at BM29 (ESRF) and preliminary analysis shows that *in vitro* the protein is in the monomeric form, as suggested by size exclusion chromatography. *In vivo* experiments suggest that MsmK is able to dimerize. Integration of these results will help to understand the enzymatic mechanism of MsmK and its importance in pneumonia development.

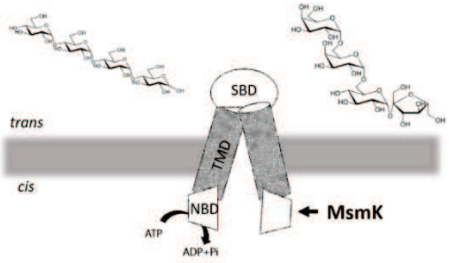

Fig. 1: Illustration of an ABC importer energized by MsmK with sugars that are possible importers (maltotetraose and stachyose respectively). In the scheme, SDB represents the substrate binding domain, TMD the transmembrane domain and NBD the nucleotide binding domain.

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A novel pectin binding protein from the human gut microbiota

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Keywords: Human Microbiota; Protein-Carbohydrate interactions; Glycan Microarrays; X-ray crystallography;

The human gut microbiota is a highly carbohydrate-active microbial community with a broad capacity to metabolize dietary and host-derived glycans, a key feature to keep nutritional balance and modulate the immune system[1]. Thus, understanding carbohydrate recognition in the gut is of utmost importance for human health.

Prominent microbiota strains, such as *Bacteroides thetaiotaomicron*, exhibit extensive sets of substrate-specific genes that allow bacteria to cope with nutrient fluctuation. Each set encodes all the necessary elements for the recognition and degradation of a specific glycan, including carbohydrate-active enzymes (CAZymes) associated with carbohydrate-binding modules (CBMs)[2]. In the genomic Era, there is an urgent need to apply high-throughput (HTP) approaches to study these recognition systems at a functional and structural level.

Here, we report an integrative strategy to characterize human microbiota carbohydrate-recognition systems, combining HTP protein production, ligand discovery using carbohydrate microarray technology[3] and structural characterization of new protein-ligand complexes with X-ray crystallography[4]. We highlight our findings on a newly identified CBM - BT0996C, from a *B. thetaiotaomicron* CAZyme involved in the degradation of the most complex plant pectic polysaccharide known, rhamnogalacturonan-II[5]. BT0996C was recombinantly produced and its specificity assigned to pectic carbohydrates. The attempts of protein crystallization produced crystals diffracting at 1.4Å resolution (*C2, a=98Å, b=47Å, c=287Å* and β=*92Å*; twinned; ESRF, ID30A data collection) and at 3Å resolution ($P4_3$, *a*=*b*=49Å and *c*=284Å; in house Cu Kα data collection). Moreover, the low identity with known proteins and incomplete data sets prevented the full assignment of the model by molecular replacement. Crystallization optimization trials are ongoing. The structure elucidation of novel CBMs, such as BT0996C, impacts on understanding microbiota metabolic capabilities and their symbiotic relationship with the human host.

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Structural-functional characterization of a new chitin-binding protein module from *Clostridium thermocellum*

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Keywords: *Clostridium thermocellum*, carbohydrate-binding module, LysM domain, carbohydrate microarrays, X-ray crystallography

Plant and fungal cell-walls are constituted by recalcitrant polysaccharides with diverse sequences that present enormous potential in biotechnology applications[1,2]. Several anaerobic microbial organisms have evolved an extracellular multi-protein complex, the Cellulosome, composed of modular Carbohydrate Active enZymes (CAZymes) and associated non-catalytic carbohydrate-binding modules (CBMs), which efficiently contribute for the biodegradation of recalcitrant polysaccharides[2,3]. The CAZy database (http://www.cazy.org) organizes the identified CBMs by sequence similarity into different families. Deposition of CBM sequences in the CAZy database is continually growing for which characterization and structure-function analysis is essential. Remarkably, certain CBMs are not associated with the cellulolytic system and can also act isolated. For instance, LysM domains, which display binding to several types of peptidoglycan and chitin, have important roles in signalling and symbiosis between bacteria and plants[4].

Our strategy to identify CBMs with relevant binding properties, makes use of carbohydrate microarray analysis for ligand discovery[5]. The results were complemented with ELISA and Isothermal Titration Calorimetry (ITC) assays and ligand-specificity was further assigned by analysis in a sequence-defined microarray. In *Clostridium thermocellum*, our analysis revealed that CBMs assigned to family 50 are highly specific for β-1,4-linked-*N*-acetyl-glucosamine (GlcNAc), with a minimum degree of polymerization of 3 residues. Protein crystals in complex with the ligand were obtained and the structure was solved by X-ray Crystallography to 1.5Å resolution. The mode of binding is under further study by molecular docking and molecular dynamics simulations. This information will allow to understand the mechanisms of carbohydrate-recognition by these CBMs and contribute to elucidate their role.

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Structural and biochemical insights into R2TP complex

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Keywords: Cancer, Molecular Machine, AAA+, R2TP

Human R2TP complex composed by RuvBL1, RuvBL2, RPAP3 and PIH1D1, plays an important role in the assembly of other protein complexes such as RNA polymerase II, snoRNP's, PIKK signalling, and in apoptosis[1]. So far, the only full-length structures available belong to RuvBL1 and RuvBL2, which can assemble not only into homo-hexamers, but also in a hetero-oligomeric way with alternating RuvBL1/RuvBL2[2].

Through X-ray crystallography at ESRF synchrotron, our group has solved the ring-shaped form of RuvBL1 and RuvBL2, typical for the AAA+ protein family to which RuvBL's belong. The structures revealed a protruding domain II from the ring, which is a unique domain among AAA+ believed to bind both DNA and proteins[2-3].

Since R2TP has been related to hepatocellular, renal and gastric carcinomas[4], understanding how it assembles its different targets can have an impact on future cancer treatment approaches.

Our group will study the interaction of R2TP with its biological partners using Surface plasmon resonance, in parallel with X-ray crystallography and Cryo-EM to solve the protein structures, and further understand what the ATPase activity of RuvBL's does in the R2TP context.

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A XANES study of the Sn *K*-edge in slag by-products from tin smelting experiments

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Keywords: XANES; Tin; Slags; Archaeometallurgy

Tin was a very important alloying element in Western Europe in the production of bronze (Cu-Sn alloy) since the second millennium BC (Bronze Age), when most metallic artefacts were made of this alloy.

Smelting experiments using cassiterite collected in the NW Iberian territory were made to produce tin in a very simple and small scale manner, using a small open pit structure to reproduce what could have been the manufacturing process of tin in prehistoric times.

Chemical and structural analysis of the products by XRF, SEM-EDS and XRD were made to achieve a detailed knowledge of the characteristics of the materials [1]. Additionally, an X-ray absorption near-edge structure (XANES) study was performed on three types of slags previously identified (Type 1, Type 2 and Type 3) to obtain information on the oxidation state of Sn. The analyses were made at the European Synchrotron Radiation Facility (ESRF) at the SpLine beamline BM 25A (5-45 keV).

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X-ray and spectroscopic characterization of a peroxidase from the extremophile *Deinococcus radiodurans*

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Keywords: Dye-decolorizing enzyme; Oxidative Stress; Crystallography; Spectroscopy;

Our cellular macromolecules are continuously damaged by harmful reactive oxygen species (ROS) which are produced as natural by-products during intracellular metabolism (respiration) and by external sources (e.g. UV-irradiation and cigarette smoke). If these damages are not prevented and/or repaired, they promote oxidative damages, which ultimately can lead to bacterial cell death[1]. Fortunately, the presence of redox scavenging systems and DNA repair machineries reduces the extent of induced damages; however the underlying molecular mechanisms of these systems are largely unknown[2]. Here we describe the cloning and expression tests of one peroxidase – Dye decolorizing peroxidase - from the extremely radiation and desiccation resistant bacterium *Deinococcus radiodurans*. The enzyme was overexpressed in *E. coli*, purified, crystallised and characterized by UV-vis and Resonance Raman spectroscopy. The obtained crystals were used for data collection in DLS (Oxford) and diffracted to 2.2 Å resolution. Structure determination by molecular replacement, using previously determined ~2 Å resolution DyP structures with ~30% sequence identity as templates, are ongoing. Our aim is to perform structure/function analysis of this protein and determine its potential role in the antioxidant defense system of *D. radiodurans.*

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Recombinant production and crystallization of a novel marine bacterial collagenase

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Keywords: Expression optimization, Protein purification, Periplasmic protein

Collagenases are collagen-degrading enzymes, which have a high potential for applications in feed, pharma and cosmetic industries [1]. The use of bacterial collagenases has gained increasing interest in these areas, due to their broad substrate specificity [2]. However, these enzymes are still relatively unknown and the discovery and characterization of new collagenases could benefit their potential application [1,3]. A particular case where bacterial collagenases can be applied is the fish industry, where a vast amount of fish skin is leftover after slaughtering of the fish for human consumption. This fish skin could be converted into collagen peptides by the action of collagenases and further be used in facial creams, as nutritional supplements and in animal feed industry.

The work presented herein focuses on the study of a novel collagenase from a marine bacteria: collagenase x. In order to obtain this protein for characterization experiments, the sequence of the collagenase was synthetized (optimized for expression in *E. coli*) and inserted into the pET-22b(+) vector, which contains a pelB signal sequence for periplasmic export, as well as an HisTag sequence to facilitate purification. Small-scale expression tests were performed with three different *E.coli* strains and BL21 (DE3) was selected for further tests due to its high expression level. Medium-scale test expressions were further performed at different temperatures and induction times, in order to select the best condition for large scale protein production and periplasmic export. Protein purification was performed by affinity chromatography (HisTrap) and gel filtration and the selection of an optimal protein buffer was achieved with thermal shift assays. Initial crystallization experiments resulted in formation of small crystals, which are currently being optimized for data collection.

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Structural and functional studies of a new class of *Mycobacterium tuberculosis* inhibitors

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Keywords: Tuberculosis, *Mycobacterium tuberculosis*, Protein tyrosine phosphatase A (PtpA), Chalcones, X-ray crystallography, Biophysical and enzymatic methods

Tuberculosis (TB) is one of the oldest and deathly infections affecting mankind.[1] *Mycobacterium tuberculosis* (Mtb), an intracellular aerobic pathogen, is the main causative agent of TB affecting preferentially the pulmonary system.[2,3] The survival of Mtb in its host is directly related to the release of protein tyrosine phosphatases (PTPs) since these proteins interfere in macrophage cell signalling (Fig. 1).[4] Protein tyrosine phosphatase A (PtpA) has been shown to play a crucial key role in this process being a promising target for the development of anti-tuberculosis drugs.[5] The crystal structure of the native protein was solved in 2005.[6]

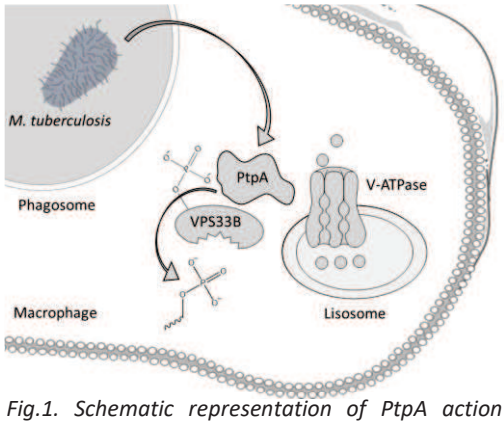

Fig.1. Schematic representation of PtpA action mechanism. PtpA is secreted by Mtb to cytosol macrophage, during infection, where the substrate, protein VPS33B, is located at the phagosome-lysosome fusion interface. Substrate desphosphorilation affects phagosome maturation, since it blocks V-ATPase recruitment to phagosome.

Among several tested compounds, chalcones have been identified as potential competitive inhibitors of PtpA. Preliminary studies revealed that the inhibitor's predominant factor is the molecule planarity/hydrophobicity and the nature of the substituents that establish hydrogen bonds with the residues in the active site of PtpA.[4,7]

In this work, the inhibitory properties of six chalcones are under study by a combined biophysical and structural approach. PtpA was successfully overexpressed and purified and it was used in enzymatic and thermal shift assays. In parallel, crystallization trials were conducted both by soaking and co-crystallization techniques and a complete dataset was collected at 2,9Å resolution (beamline ID23-2, ESRF). The structure was solved by MR methods in P6 space group. Structural analysis is still in progress, but preliminary analysis of the electron density map suggests the binding of the inhibitor to PtpA active site.

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Use of synchrotron radiations to probe particle orientation in electrically conductive nanocomposite under uniaxial strain

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Keywords: Additive manufacturing, Polycaprolactone, multiwall carbon nanotubes, nanocomposites, electrical conductivity, strain, anisotropy.

Nanocomposites are essentially nano dielectrics containing a dielectric polymer as the matrix and a low level of nanoparticles. The nanoparticles are conductive and have ability to pass an electric current when there exists a network of such particles within polymer matrix. Carbon nanotubes are used to form percolative networks in a polycaprolactone matrix such that the sample behaves as a bulk conductor. Polycaprolactone filled with different contents (0.5 wt% and 1.0 wt %) of multiwall carbon nanotubes was investigated in X-ray synchrotron experiments simultaneously with electric conductivity measurements under uniaxial deformation. As the material system is subjected to strain, some of the conductive pathways may be disrupted and new pathways may form. This happens because of the reorientation of the crystalline phase, amorphous phase and the multiwall carbon nanotubes under the effect of uniaxial deformation. Small angle x-ray scattering technique reveals useful information about these phases that can help to design or select correct material for flexible smart electronics. Moreover, it is possible to probe the structure and dynamic behaviour of the nanostructure in real time in such type of novel experiments. We have performed the measurements using the NCD-Sweet beam line at the ALBA synchrotron facility in Barcelona, Spain. A portable experimental set-up has been established to facilitate such measurements where it can be mounted on a synchrotron beam line to enable small-angle x-ray scattering measurements to be made during the deformation cycle. The poster describes the methodology, 3D printing process of strands and x-ray scattering of these novel experiments. We use the data to develop a model of the behaviour of these elastomeric nanocomposites. This builds on previous work on the electrical properties of conductive elastomers [1, 2].

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Sb and Pb speciation through XANES in 18th to 19th century Portuguese glazes

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Keywords: XANES, Cultural Heritage, Glazes, Lead and Antimony

Speciation of fuser metals and colorants in ancient tile glazes allows for tracing chemical affinities and correlations in phase behaviour. This is the case of antimony-containing pyrochlore-type double oxides such as bindheimite, a yellow pigment with approximate formulae $Pb_2Sb_2O_7$.

To ascertain antimony speciation and to better understand pigment incorporation in ancient glazes and ageing mechanisms it is necessary to study both antimony and lead speciation in the glaze (glassy matrix and intensively coloured domains). Accordingly, the XAFS characterization of both Sb *K*-edge and Pb *L*-edge is the optimal methodology.

Samples analysed are unique majolica-type polychrome high relief tiles from 19th century Portuguese manufacture, precursors of the Art Nouveau period in Portugal, from the UNESCO World Heritage Pena National Palace (Sintra, Portugal). They were chemically and structurally characterized using XRD, µ-PIXE, µ-Raman, Optical Microscopy and VP-SEM [1]. Here reported are the first results on XANES analysis for the clarification of the structural role of Sb and Pb in the glazes for comparison with results from 17th century tile glazes [2]. The detailed structure of an absorption edge (XANES) is the answer on information on the bonding state and speciation of an element responding also to the geometry of the atomic environment. XANES has been successfully applied to this type of studies for other type of tile glazes and glasses being the answer to questions of this specific majolica-type glazes [2-6].

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Co *K* edge XANES in ceramic pigments form faience and porcelains from the 16th to the 18th century.

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Keywords: XANES, Cultural Heritage, Glazes and glasses, Cobalt

An important set of historical samples from three classified national monuments were characterized by our group using several laboratory techniques such as XRD, μ-PIXE, μ-Raman, μ-XRF, Optical Microscopy and VP-SEM [1-3]. The samples are glazes from the National Palace of Sintra, lustres form the Fronteira Palace in Lisbon, and porcelains belonging to the Santa Clara Monastery in Coimbra. The glazes display a silica-lime-alkali glass with the addition of low melting point metals and chromophores based on Co and Cu for the blue colouring. In lustres the colourless glaze has a lead-alkali silicate composition and a copper-rich lustre overlay. From Coimbra the blue and white porcelains have Co as the colouring agent in the blue inlays.

To achieve a comparison between manufacturing techniques, pigments used, conservation status and origin of the pieces several fragments were characterized using X-Ray Absorption Spectroscopy. XAS techniques can provide information on the structural behaviour of transition metals in the vitreous matrix - namely, their bonding state and coordination environment, that may also configure the cause of increased glaze instability along time and of final collapse due to the action of external environmental agents.

First results of the speciation state of Co in the different historical fragments using XANES will be presented in an attempt to correlate and complement other measurements in Co and other transition metals in blue-and-white glazes from different periods of tile production, along with other vitreous coatings over ancient ceramics such as lustres and porcelains [4-6].

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Local structure of indium in transparent Ga-In-Zn-O based amorphous thin film semiconductors: XANES at the In *K*-edge

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Keywords: indium, *K*-edge, XAFS, semiconductors

Discovered in 1863 and isolated four years later as a metallic element, indium is widely used in various technological fields (e.g. electronics, low melting-temperature alloys, solders) and lately it became relevant for the production of devices based on innovative nanotechnologies.

New research areas have been opened to the thin-film transistor (TFT) field with the emergence of the first semiconductor oxide-based transistors in 1996 [1]. Later new advances were made through oxide semiconductor TFTs based on ZnO [2,3] or ZnO compound materials [4]. Their remarkable electrical properties, comparable to or even better than amorphous silicon (a-Si) TFTs, can propitiate potential fields of application such as flexible and transparent devices.

Results concerning transparent transistors containing indium with reduced processing temperatures and/or enhanced electrical properties in comparison with the first ZnO TFTs encouraged the exploitation of new multicomponent oxide semiconductors based on heavy-metal cations, e.g., zinc–tin oxide (ZTO) [5], indium–zinc oxide (IZO) [6,7], and gallium–indium–zinc oxide (GIZO) [8,9], which have excellent electrical properties in spite of their amorphous structure, something that is unusual for conventional covalent semiconductors.

To design such amorphous semiconductors, knowledge on the local structure around the cations and the origin of the unique carrier transport properties are of utmost importance. Thus, it is essential to understand the local coordination and electronic structure of cations in these amorphous semiconductors. First results obtained will be shown concerning the probing of the speciation state (coordination plus valence state) by X-ray absorption spectroscopy at the In *K*-edge.

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Growth and characterization of Ti–Al–N thin films

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Keywords: magnetron sputtering deposition, hard coatings, structural evolution, *in-situ* x-ray diffraction, synchrotron radiation

The development of the preferred orientation of magnetron-sputtered $Ti_{1-x}Al_xN$ during the growth process has been analysed by X-ray diffraction. *In-situ* investigations performed with a two-magnetron sputter deposition chamber mounted into the six-circle diffractometer of the synchrotron radiation beam line ROBL at the European Synchrotron Radiation Facility are presented. Low Al concentration $Ti_{1-x}Al_xN$ thin films were deposited by reactive cosputtering from Ti and Al targets onto oxidized Si(100) wafers. Off-plane and in-plane x-ray diffraction data were recorded and the measurements were supplemented by *ex-situ* cross-sectional transmission electron microscopy analyses. For high deposition rates the crossover from initial (001) to final (111) preferred orientation is observed, which has been often reported for TiN. Reducing the deposition rate by increasing the N_2 partial pressure leads to a more dense film with an almost complete (001) preferential orientation, practically independent of substrate temperature and Al concentration. The microstructural evolution below the segregation regime can be understood within current thermodynamic and kinetic models for competitive growth processes developed for pure TiN with their competing energetic balance and diffusion behaviours.

O_2 access to the active site of the [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* Hildenborough

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Keywords: hydrogenase, high-pressure, oxygen tolerance, X-ray crystallography

Hydrogenases catalyse the reversible oxidation of hydrogen into two electrons and two protons and are of great potential economic interest because they can produce hydrogen from biological sources or they can be used to convert hydrogen into electricity. While many hydrogenases are rapidly inactivated by oxygen, the [NiFeSe] Hydrogenase from D. vulgaris Hildenborough is an oxygen-tolerant enzyme, i.e., it can function for a longer time in the presence of small O_2 amounts. However, the enzyme activity is affected by O_2 via two structural modifications: one of the Cys ligands of the Ni atom in the active site is irreversibly converted to sulfinate, and the proximal iron-sulphur cluster is reversibly oxidized to a form containing 2 oxygen atoms (Marques *et al.*, 2013; Marques *et al.*, 2017).

With the aim to protect the Cys ligand from O_2 attack, several hydrogenase variants were produced using a homologous expression system developed in our labs. To obtain structural information that could assist in the design of additional variants, hydrogenase crystals were incubated with O_2 under high-pressure (more than 75 bar) for varying periods of time at the High-Pressure Lab of the ESRF, and data collected at ESRF beamlines ID23-1 and ID30B.

Surprisingly, the location of the O_2 molecules in the hydrogenase crystal structures proved to be non-trivial, and it was realized that the intense X-ray beam used under normal data collection conditions lead to the reduction of the O_2 molecules by photoelectrons produced in the crystal by the beam.

This problem was circumvented by collecting a dataset with very low X-ray dose absorption by the crystal and comparing the resulting electron density map with another obtained from a dataset recorded with a normal X-ray dose as suggested by the beamline software.

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The use of time-resolved in-situ small-angle x-ray scattering techniques to evaluate the molecular organisation changes which accompany 3d printing of polymers

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Keywords: 3d printing, manufacturing, polymer processing, properties, tissue engineering, functionally graded materials

3d printing is a procedure which forms part of the family of direct digital manufacturing technologies which enable objects to be produced directly from a digital definition without the need for specialised tooling or moulds. Here we focus on the use of 3d printing to produce plastic parts. Essentially a thin strand of molten polymer is extruded on to a moving build platform in a defined manner to build up a structure layer by layer. As with any polymer processing technology, the process conditions will influence the polymer morphology and structure on cooling which in turn will impact on the properties. As part of a major project to full understand all aspects of 3d printing we have performed Small-angle X-ray Scattering experiments on parts prepared in this manner. We have design and built a 3d printer which can be mounted on the ALBA NCD-Sweet beam line so we can follow the structural development in real-time. We show how we have used preliminary experiments to help optimise the design of the 3d printer to be mounted on the beam-line. We show examples of how the printing parameters affect the structure and morphology. We describe the experiments to be performed later this year which will test the efficacy of this approach and we discuss the type of information we hope to extract. We show how the results we obtain can be used to optimise the 3d printing technology and the materials used. We give examples of the impact on properties.

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Biochemical and Structural Studies of Human Aldehyde Oxidase for Efficient Drug Development

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Keywords: Drug metabolism, Aldehyde oxidase, Single nucleotide polymorphisms, X-ray crystallography, ThermoFAD

Aldehyde oxidase (AOX) is a cytosolic enzyme, predominantly expressed in liver, involved in Phase I metabolism of numerous drugs and xenobiotics. The true physiological function of AOX is unclear but it possesses a broad substrate specificity and performs diverse reactions, including oxidations (e.g. aldehydes and aza-heterocycles), reductions (e.g. nitro, S- and N-oxides) and hydrolysis of amide bonds[1].

The number and types of active AOX genes varies according to the animal species considered. Humans and higher primates have a single functional AOX1 gene while rodents are endowed with four different active genes[2]. As a result, the expression levels and prevalent isoforms of AOX in humans and in animals are different, leading to the lack of a suitable model for human metabolism prediction. Moreover, besides the cross-species different heterogeneity, single nucleotide polymorphisms (SNPs) of hAOX have been reported as affecting the ability of the enzyme to metabolize different substrates[3]. The relevance of AOX in the metabolism and clearance of new drugs, along with the high interindividual variability observed in AOX, calls for a better understanding of the catalytic properties, substrate specificity and inhibition mechanisms.

We applied the high-throughput biophysical approach ThermoFAD in order to characterize the impact of hAOX SNPS in terms of thermostability and correlated it with biochemical and structural data. In addition, we solved the structure of new hAOX-inhibitor complexes[4]. The structural analysis of these complexes revealed new inhibitor binding sites structurally conserved among mammalian AOXs. All X-ray diffraction data were obtained in the structural biology beamlines of ESRF (Grenoble, France) and SLS (Villingen, Switzerland). The results obtained are of great interest for the study of AOX in clinical drug interactions and for the rationale design of future AOX stable putative drugs and inhibitors.

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Impact of interface Non-synonymous Single Nucleotide Polymorphism (nsSNPs) on Bcl-2:Bax interaction, a computational perspective

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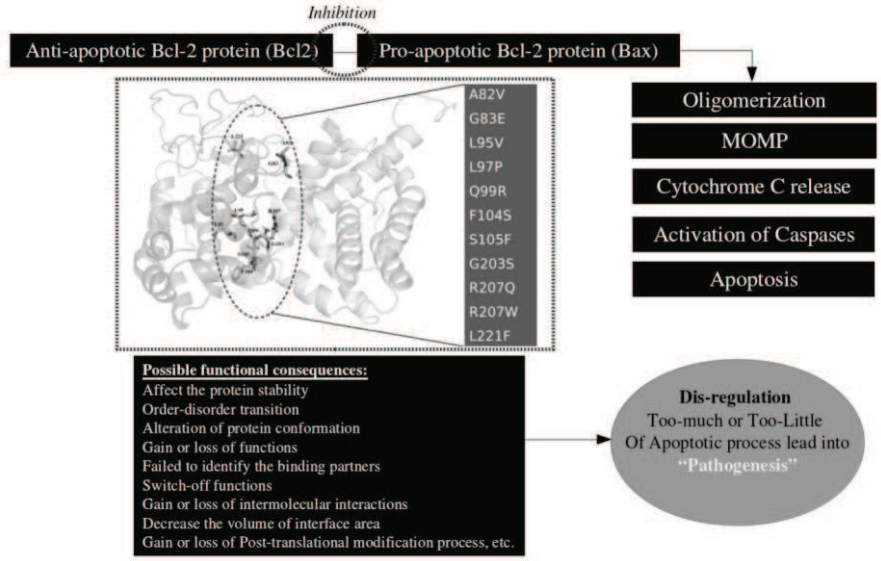
Keywords: Apoptosis, Bcl-2, Bax, Protein-Protein docking, MD simulation

Apoptosis or Programmed Cell Death (PCD) is an essential component of various biological events namely normal cell turnover, embryonic development, proper development and functioning of immune system, etc [1]. Bcl-2 family of proteins regulates the PCD mechanism by either induction (pro-survival) or inhibition (pro-death) maintaining the balance between Pro-apoptotic (Bax and Bak) and Anti-apoptotic (Bcl-2, Bcl-w and Bcl-xL) members [2]. Non-synonymous Single Nucleotide Polymorphisms (nsSNP) are point mutations which can alter the sequence of amino acid residues and these alterations lead to the pathogenic phenotypes. In recent years, the consequence of interface nsSNPs upon protein–protein interactions (PPIs) has also been examined, giving a greater perception into the mechanisms by which nsSNPs can lead to disease. The objective of the current study is to investigate the distribution of nsSNPs in anti-apoptotic Bcl-2 protein and understand their impact on pro-apoptotic Bax interaction, using computational methods. Our *in silico* analysis showed that R207Q interface nsSNP (Accession No: rs369294037) could be the most promising deleterious variant which affects the Bcl-2:Bax interactions significantly. This study provided strong insights to understand the impact of other inhibitors/binding partners towards Bcl-2 protein, and thus opens new direction for anti-cancer therapeutics.

Figure 1: Schematic representation of present study



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In-situ thermomechanical simulation by Gleeble® Synchrotron system on NiTi shape memory alloy

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Keywords: Ni-rich NiTi shape memory alloy, synchrotron radiation X-ray diffraction (SR-XRD), Gleeble.

NiTi alloys have attractive functional properties as shape memory effect and the superelasticity appropriated to be used in different segments. These functional properties are obtained through the thermomechanical process[1]. Thermomechanical processes aim to obtain the appropriate shape of the material, to control phase transformation temperatures, to improve the alloy characteristics, and refine shape memory effect or superelasticity. To obtain the desired microstructure the hot work should be performed to assure the mechanical and functional properties adequate[2]. In this study, the deformation behaviour of the as-cast sample Ni-rich NiTi alloy was investigated using the hot compression test at 850 ºC and with a deformation rate of 10^{-1} s^{-1}. The X-ray scattering and Thermo-Mechanical simulation (XTMS) experiment were carried out the XRD1 beamline, LNLS - CNPEM, Campinas - Brazil. The uniaxial compression tests were carried out on an advanced thermomechanical simulator, the Gleeble® Synchrotron system.

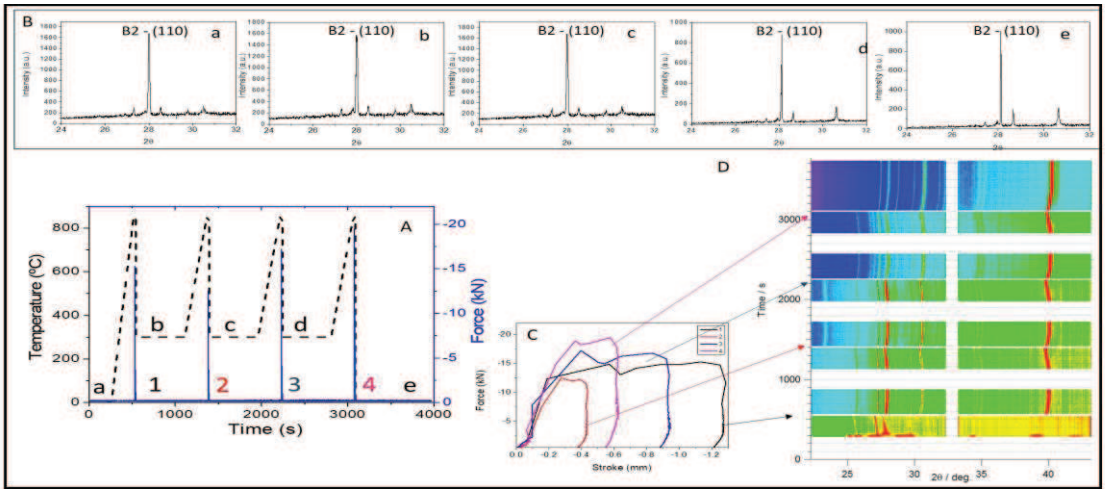

Figure 1 – Scheme of thermomechanical simulation

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Stress-assisted aging of NiTi shape memory alloy

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Keywords: synchrotron radiation, in situ X-ray diffraction, shape memory alloys (SMA).

The NiTi alloys have the unique shape memory effect and superelasticity characteristics which make them attractive functional materials. For this investigation, a sample of 50.8 at.% Ni-Ti in the as-cast condition was used [1]. The sample was compression aged at 500 ºC for 30 min after hot deformation at 850 ºC. In situ deformation experiment was conducted in a modified dilatometer DIL-805 (Bähr) at the HZG beamline (HEMS/P07-EH3, Petra III, DESY, Hamburg). The Ni_4Ti_3 precipitation affects the functional and structural properties of these alloys [2,3,4]. In this study, we observed the Ni_4Ti_3 stress-assisted precipitation, using synchrotron radiation-based X-ray diffraction (SR-XRD). Transformation temperatures were increased after aging, but the material still shows superelastic behaviour at room temperature, as required by the orthodontic applications aimed for this material.

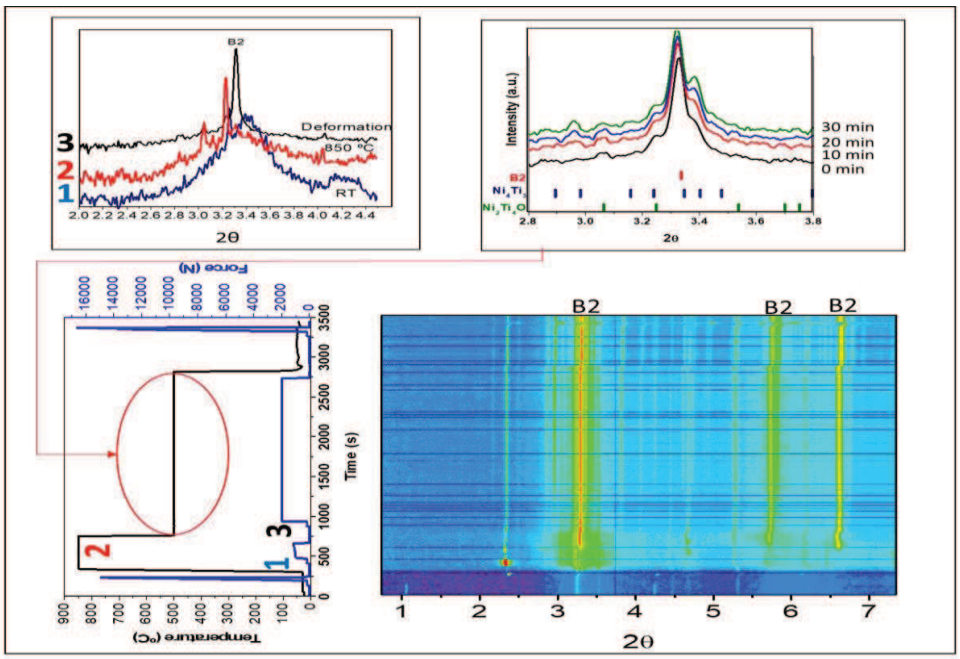

Fig. 1 – In situ XRD during hot deformation and stress-assisted aging of Ni-Ti SMA.

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Structure of two mutated Endonuclease III from *Deinococcus radiodurans*

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Deinococcus radiodurans is an extremely radiation and desiccation resistant bacterium, which can withstand 200 times higher doses of ionizing irradiation than other bacteria without losing viability [1]. The resistance mechanism is not known, but an efficient DNA repair machinery is considered to play a key role in it [2]. Endonuclease III (EndoIII) is an ubiquitious bifunctional enzyme which belongs to the helix-hairpin-helix family of DNA glycosylases, with an [4Fe-4S] cluster. It has specificity for a broad range of oxidized pyrimidines lesions, removing numerous forms of damaged bases from DNA [3]. It has previously been performed structure/function analysis of three EndoIII enzymes from D. radiodurans, EndoIII1, 2 and 3, which showed that EndoIII1 and 3 possess unusual properties compared to previously EndoIII enzymes from other organisms [4]. The structural analysis revealed that some specific amino acid substitutions close to the active site could explain the observed alternative activities. Here we are presenting the crystal structures of two mutated Endonuclease III enzymes from this organism and their biochemical characterization to analyse the effect of the mutated amino acids (X-ray diffraction data were collected on BL13-XALOC beamline at the ALBA Synchrotron (ALBA; Barcelona, Spain).

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Mechanisms of response to Nitrosative and Oxidative stress in *Clostridium difficile*

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The presence of considerable amounts of oxygen in the atmosphere forced modifications in the cellular metabolism of organisms 1. To overcome this problem, organisms have found ways to convert both reactive oxygen (ROS) and NO (RNS) species through the synthesis of enzymes responsible for their detoxification1. One of the common aspects of these enzymes is the presence of Fe centers responsible for the conversion of these species to harmless molecules in organisms.
Clostridium difficile is an anaerobic organism that contains in its genome, enzymes that are possibly involved in the response to oxidative and nitrosative stress.2,3
The aim of this study was to investigate the role of the enzyme Flavodiiron protein (FDP) in the response to O2, No and H2O2 as well as their own interaction.
This protein was biochemically and spectroscopically characterized using UV-VIS and EPR spectroscopies, while the reactivity towards O2 and NO were analysed by amperometric methods. The structure will be obtained by X-ray crystallography, as crystals were already produced.
At this point, it is possible to conclude that these enzymes are able to reduce both substrates.

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New structural insights on protein-$V^{IV}OSO_4$ and protein-$NaV^{V}O_3$ interactions revealed by X-ray crystallography and SAXS

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Keywords: Vanadium and medicinal chemistry, $V^{IV}OSO_4$, $NaV^{V}O_3$, Human serum transferrin, Hen egg white lysozyme, X-ray crystallography, Small Angle X-ray Scattering (SAXS)

Vanadium is an important element with different biological functions.[1] Moreover, the therapeutic use of vanadium – inorganic and complexed with small organic ligands – has been also suggested and several studies on the topic are available in the literature.[2,3,4] Herein, we present a structural study focused on the interactions of two inorganic vanadium compounds ($V^{IV}OSO_4$ and $NaV^{V}O_3$) with different proteins: human serum transferrin (HTF) and Hen egg white lysozyme (HEWL).

HTF is known as an important metal ion blood carrier and Small Angle X-ray Scattering (SAXS) was firstly used to confirm the protein-ligand binding.[5] Three datasets – native apoHTF, apoHTF-$V^{IV}OSO_4$ and apoHTF-$NaV^{V}O_3$ – were collected at beamline BM29 (ESRF, Grenoble, France). Significantly different parameters have been determined suggesting that both compounds – particularly vanadate(V) – can effectively interact with HTF. In fact, the results indicate a partial closing of apo-HTF upon binding of V^{IV} and V^{V} which is less pronounced that the one caused by the Fe^{III} ion.

To further characterize such interactions, soaking experiments with HEWL were performed and a 1.34Å resolution HEWL-$V^{IV}OSO_4$ structure was obtained at beamline BM30A (ESRF, Grenoble). The structure reveals the presence of three metal adducts next to Asp52, Asp87 and Leu129 (Figure 1). The adducts were properly modeled and different occupancies and geometries have been obtained. Importantly, the detected V^{IV}=O bond distances appears to confirm the oxidation state of vanadium.

In conclusion, this work proves that $V^{IV}OSO_4$ and $NaV^{V}O_3$ may be transported in blood by HTF. The obtained insights are important to properly evaluate the pharmacokinetics of the compounds contributing for their putative use as safe drugs.

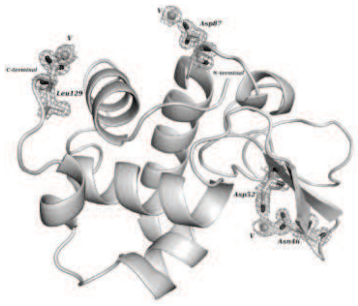

Figure 1 – Overall representation of the HEWL-$V^{IV}OSO_4$ structure.

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New opportunities to explore Cyclophilin D as an effective therapeutic drug target

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Keywords: Multiple sclerosis, Fragment-based lead discovery, Protein crystallization, Cyclophilin

Cyclophilin D (CypD) is a key regulator of the mitochondrial permeability transition pore. Mitochondrial dysfunction has been implicated in a cascade of cellular processes leading to multiple sclerosis and cardiovascular disease [1], making CypD an attractive drug target.
Three different inhibitor/hit series including urea derivatives were discovered at Merck KGaA (Germany) using fragment-based SPR-screening and medicinal chemistry optimization. However, from the 58 SPR-confirmed hits, only 6 CypD-fragment crystal structures were obtained. Moreover, these hit series did not show sufficient cellular potency together with optimal pharmacokinetic properties and the project was abandoned by Merck. Until now, CypD wild-type failed all attempts at crystallization. In 2005, protein engineering on the enzyme surface was performed by Schlatter et al. [2] and the K175I mutant yielded crystals that diffracted to 1.7 Å resolution. The CypD K175I mutant was used in the previous work performed by Merck KGaA and partners.
In this work, two different mutants of CypD (*hs*CypD (K175I) and a double mutant *hs*CypD (K167Q, K175I)) are used to revisit the previous Merck hits that failed to co-crystallize or led to crystal structures with unfavorable crystal packing. Furthermore, a new "dry" co-crystallization technique [3] is being used to allow the preparation of protein co-crystals avoiding DMSO presence. Successful expression, purification and crystallization of both CypD variants was already achieved and their structures determined. More recently, crystallographic structures of both variants where Merck fragments are present are being obtained (with ESRF, ALBA and DLS synchrotron beamlines usage) and these new structures will be used to feed a new structure-based design loop, leading to the synthesis of new compounds within a final goal of designing a better ligand/more potent inhibitor of CypD.

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X-ray absorption near-edge spectroscopy (XANES) applied to selenium speciation study of incrustations from Fogo volcano, Cape Verde

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Keywords: XANES, Se speciation, Fogo volcano, incrustations, Cape Verde

Selenium, an element that usually substitutes sulphur, assumes a variable speciation state depending of the carrier mineral. This fact allied to the high concentration obtained for this element in fumarole incrustation samples from Fogo volcano (Cape Verde) plays a major health hazard concern as local populations use sulphur and white materials as treatment for some diseases. Mineral phases identified by X-ray diffraction in incrustations that resulted from the last eruption, were e.g. native sulphur (α-S), anhydrite ($CaSO_4$), bassanite ($CaSO_4.1/2H_2O$), gypsum ($CaSO_4.2H_2O$), thenardite (Na_2SO_4), ralstonite ($Na_xMg_xAl_{2-x}(F,OH)_6.yH_2O$).

To clarify the speciation state of Se and the nature of Se-carrier phase(s) on incrustations samples, an X-ray absorption spectroscopy study (XANES) using synchrotron radiation was undertaken at Se *K*-edge (ESRF, beamline BM 25A), preceded by the chemical constitution study through energy dispersive X-ray fluorescence (EDXRF). The semi-quantitative analysis was previously obtained through X-ray fluorescence spectrometry with wavelength dispersive system (XRF-WDS) at the laboratory.

Different situations for Se speciation were observed: Se^{6+} tetrahedral, in a mixture of bassanite and anhydrite; Se^{4+} pyramidal coordination in ralstonite samples; and Se^0 in a sulphur sample. The highest content obtained for selenium was 1000 ppm (1mg/g) in the sulphur sample indicated that selenium is mainly carried by sulphur due to diadochic replacement of S by Se. Being 0.3mg/day the tolerable upper intake level, overexposure to Se in the diet leads to gastrointestinal upsets, hair loss, white blotchy nails, fatigue, irritability, and mild nerve damage. A more complete chemical study by EDXRF and the ascertaining of the speciation state of arsenic, thallium and lead (elements also present in incrustation samples and potentially toxic) are foreseen.

From yellow, brown to blue/greyish limestone: a Fe *K*-edge study through XANES

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Keywords: Limestone, Fe *K*-edge, XANES, blue/greyish colour, Blue *Valverde*

The called "Azul Valverde" is one of the most relevant blue/grey limestones from Portugal, belonging to the Estremenho Limestone Massif (Maciço Calcário Estremenho - MCE) region, central sector of the Lusitanian Basin. MCE is a geomorphological unit where large rocks from the Jurassic age arise. Particularly, blocks for the Middle Jurassic have been the target of intense exploration for ornamental purposes. The variety "Azul Valverde" (blue Valverde) appears at the "Covão Alto" area/quarry [1], where colour variation between blue/greyish (which confers a higher commercial value to the rock) and yellow/beige associated with fractures, are observed.

Recent studies assumed that the bluish colour origin is due to the presence of dispersed organic matter that was introduced in the rock prior to early carbonate cementation processes by percolation of hydrocarbon-rich fluids; accumulation of insoluble residues were found, together with pyrite (FeS2) and darker compounds like oxides/hydroxides not identifiable by microscopic observation, being the rock a quite homogeneous calcarenite, very compact and almost completely cemented by carbonate [2].

An X-ray absorption spectroscopy study (XANES) using synchrotron radiation was undertaken at Fe K-edge (ESRF, beamline BM 25A) with the purpose of ascertain the speciation state of Fe in the three different zones of the limestone (yellow, brown and blue/greyish), in order to complement the performed studies. The details on the pre-edge region of the XANES spectrum (corresponding to the 1s→3d transition) have long been recognized as being mostly sensitive to the electronic structure and geometry of the iron site, thus the deconvolution of the pre-edge structure into pseudo-Voigt components using the program Fityk to derive the height and position of energy components, was used to interpret the still unclear colour variation due to iron in limestones. Clear differences between the three zones were found, suggesting the presence of 6-coordinated Fe3+ (goethite?) in the brown area, 6-coordinated Fe3+ or Fe3+plus Fe2+ in the yellow (limonite) and 6-coordinated Fe2+ in the grey, due to the occurrence of pyrite.

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Structural insights into the regulation and inhibition of human cystathionine β-synthase

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Keywords: cystathionine β-synthase, hydrogen sulfide metabolism, X-ray crystallography

Human cystathionine β-synthase (CBS) is a key enzyme in sulfur metabolism and plays a physiological role in homocysteine and hydrogen sulfide (H_2S) metabolism [1]. CBS exists as a homotetramer, each subunit consisting of three domains: an N-terminal heme-binding domain, a central catalytic domain harboring a pyridoxal 5'-phosphate active site, and a C-terminal s-adenosyl-L-methionine (AdoMet)-binding domain. Mutations in the *CBS* gene are responsible for classical homocystinuria, an inherited error of metabolism. CBS is also linked to several other human pathologies, such as cardiovascular disease and cancer. CBS structure and regulation are key elements to understand dysfunction and developing new treatment options for patients. The regulatory mechanisms of CBS are complex and still to be fully understood. With this aim, we obtained crystallographic data of truncated CBS co-crystallized with inhibitors, as well as in different redox states (Diamond Light Source, I03 beamline).

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