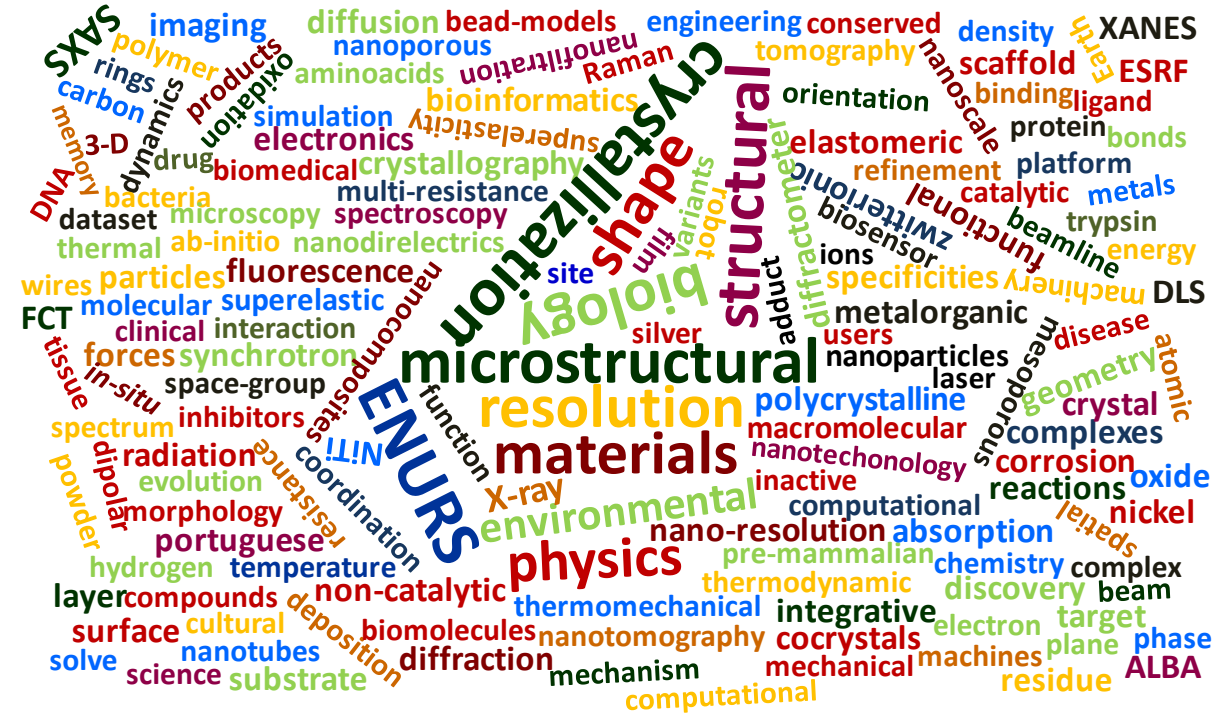
5ºENURS

ENCONTRO NACIONAL DE UTILIZADORES DE RADIAÇÃO DE SINCROTRÃO

National Meeting of Portuguese Synchrotron Radiation Users

ITQB NOVA 17 JUN 2016 | OEIRAS | PORTUGAL



Book of Abstracts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WELCOME TO THE 5th ENURS

We are pleased to welcome you to the 5th Meeting of Portuguese Synchrotron Radiation Users (5º Encontro Nacional de Utilizadores de Radiação de Sincrotrão), taking place at ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier da Universidade Nova de Lisboa, Portugal, on June 17, 2016.

The goal of this meeting is to bring together present and potential future Portuguese users of synchrotron radiation from different research fields, to share their experiences and successes with each other, to develop new collaborations and to build an even stronger synchrotron user community in Portugal.

This meeting will be of value to both current users of synchrotron radiation and to those who would like to learn more about the opportunities available for adding value to their own research programs.

While the ESRF is still the main Synchrotron Radiation facility used by Portuguese researchers and is likely to remain so in the near future, other European SR infrastructures have emerged to complement the beamline portfolio offered by the ESRF and have received an increasing number of beam time applications from Portuguese researchers.

Therefore, this year we will have the opportunity to listen to Scientists from three different European Synchrotron light sources: ALBA (Spain), DLS (UK) and ESRF (France), who will speak about the research opportunities offered by these European infrastructures.

The Meeting will provide plenty of opportunities for networking and informal discussions involving the national community and the invited SR scientists, to increase awareness on the advantages of using their infrastructures, thus strengthening our User Community.

The Organizing Committee,

Célia Romão

Pedro Matias

Program

09h30 - 10h15 Registration, Poster set-up and Welcome Coffee

10h15 - 10h30 Welcome remarks

Morning Session (Chair: P. Matias)

10h30 - 11h00 IS1 Emir Sirage (FCT) - Portugal and the LSFs in Europe

11h00 - 11h45 IS2 Caterina Biscari (ALBA) - ALBA, the Spanish synchrotron

11h45 - 14h00 ***Lunch and Poster Session***

Afternoon Session I (Chair: M. A. Carrondo)

14h00 - 14h30 IS3 Martin Walsh (DLS) - Diamond – A New Light for Life Sciences

14h30 - 15h00 IS4 Harald Reichert (ESRF) - The Next Step in the Exploitation of Storage-Ring-Based High Energy X-ray Sources

15h00 - 15h30 IS5 Gordon Leonard (ESRF) – Facilities for Structural Biology at the European Synchrotron Radiation Facility

15h30 - 16h00 IS6 Jonathan Wright (ESRF) – Looking inside materials using synchrotron X-ray diffraction

16h00 - 16h30 ***Coffee break***

Afternoon Session II (Chair: C.V. Romão)

16h30 - 16h45 O1 F.M. Braz Fernandes - In situ studies of shape memory alloys during thermal / mechanical cycles

16h45 - 17h00 O2 Ricardo Araújo **-** Unveiling the anatomy of mammal predecessors with synchrotron radiation-based micro-computed tomography: the first insights into braincase and occiput in Gorgonopsia

17h00 - 17h15 O3 Geoffrey Mitchell - Strain dependent morphological study in elastomeric nanodirelectrics

17h15 - 17h30 O4 Teresa Santos-Silva - The active-site pocket of trypsin: a dance hall

17h30 - 17h45 Closing remarks (P. Matias)

Invited Speakers

IS1

Fundação para a Ciência e Tecnologia

Portugal and the LSFs in Europe

Emir Sirage
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IS2

ALBA Synchrotron

ALBA, the Spanish Synchrotron

Caterina Biscari

Director, ALBA

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ALBA history started in the early nineties, when a group of visionary scientists proposed to build a synchrotron light source in Spain, as the first national large scientific infrastructure. It was meant to serve and help the development of the national Synchrotron Light User Community, to build up a group with expertise in accelerator physics, to develop an industrial environment with capacities of participation in similar facilities all around the world.

The green light was obtained in 2003, and the cornerstone of the first 3rd generation light source in the South-East of Europe was placed in 2006 at about 20 km from Barcelona.

Today, since the first official user in May 2012, the seven day-one beamlines have served more than three thousand users, three more beamlines are in construction, and the next phase is defined.

The talk will describe, after a short historic introduction, the present status of the infrastructure, the accelerator, the collaboration activities and the outlook for the future.

IS3

Diamond Light Source

Diamond – A New Light for Life Sciences

Martin A. Walsh

Diamond Light Source and the Research Complex at Harwell

E-mail: martin.walsh@diamond.ac.uk

Synchrotrons have now become indispensable to structural biology research and have aided the structure determination of ever more complex macromolecules including the ribosome and viruses which has been spear-headed by macromolecular crystallography. Other X-ray based techniques available to biologists at Diamond include small angle scattering and spectroscopy. Furthermore use of UV/VIS light can be exploited to aid structural and functional analysis of complex macromolecules which are largely disordered and out of bounds to crystallographic analysis by Circular Dichroism. Complementing this reductionist approach to the understanding of the workings of the living cell is the use of X-ray, electron and Infrared imaging techniques, making Diamond a unique imaging centre for cellular and structural biologists

This overview will summarize the infrastructure and techniques available for structural and cellular biology at Diamond along with planned developments that will aid the advancement of our fundamental understanding of how biological systems function.

Bullet points

- An overview of Diamond– what, where and why bother!
- Focus on Synchrotron Radiation applications in the life sciences from macromolecules to cells
- Progress towards an integrated campus for structural biology through light, X-rays and electrons

IS4

European Synchrotron Research Facility - ESRF

The Next Step in the Exploitation of Storage-Ring-Based High Energy X-ray Sources

Harald Reichert

Director of Research in Physical Sciences

E-mail: reichert@esrf.fr

The European Synchrotron Radiation Facility is Europe's premier hard X-ray synchrotron radiation source serving 45 experimental stations for public use. The facility has just finished Phase I of an ambitious upgrade programme (2009-2015) covering all aspects of the facility: photon production, experimental facilities for users, user service, and X-ray technology development. The upgrade benefits all areas of X-ray applications: Imaging, Spectroscopy, and Diffraction. A few examples will be used to demonstrate first results from the new instruments.

In parallel we have started work for ESRF-EBS project (Phase II of the upgrade programme, 2015-2022) focusing on the construction of a new storage ring with the goal to reduce the horizontal emittance by at least a factor of 30 by 2020. The associated linear increase in brilliance and coherence will enable new applications of X-rays in the study of soft and hard condensed matter. After an introduction of the main concepts behind the new source, the potential for new science will be discussed.

IS5

European Synchrotron Research Facility - ESRF

Facilities for Structural Biology at the European Synchrotron Radiation Facility

Gordon Leonard

Head of the Structural Biology Group

E-mail: leonard@esrf.fr

The ESRF's facilities for Structural Biology provide 6 undulator-based end-stations for Macromolecular Crystallography (MX) and a bending magnet beamline (BM29) for increasing popular experiments in Small Angle X-ray Scattering from solutions of biological macromolecules (BioSAXS). In this talk I will describe the facilities available and the functionality they offer, with particular emphasis on BM29, MASSIF-1, MASSIF-3 and ID30B, all of which were constructed and commissioned as part of Phase I of the ESRF Upgrade Program. I will also present ideas for how the portfolio of beamlines the ESRF provides for Structural Biology might evolve as a result of the ESRF Extremely Brilliant Source (EBS) project.

IS6

European Synchrotron Research Facility - ESRF

Looking inside materials using synchrotron X-ray diffraction

Jonathan Wright

Scientist Responsible for the Materials Science Beamline (ID11)

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We are surrounded by modern materials and devices and these have a profound impact on our day-to-day lives. X-ray diffraction can show us how atoms are arranged inside materials and this view has helped to develop the structure-property relationships which drive new materials developments. Historically, crystallographers have used either “single crystal” or “powder” diffraction, depending on the kind of sample which is available. In a useful device like a battery there may be a mixture of single crystals, powders and even liquids. We need to be able to look inside samples like these to see what is happening at the atomic scale in different places inside the device.

Synchrotron radiation sources offer high intensity X-ray beams which are extremely bright. These beams are excellent for high resolution powder diffraction which can be used with a wide range of sample environments. The instrumental resolution can be so good that particle size broadening is seen for crystallites which are over 1 micron in size. On the other hand, using focusing optics it is possible to reduce the X-ray beam size to be well below 1 micron in size so that only a few grains of a powder sample are illuminated at once.

To average over a larger number of grains for powder diffraction experiments then samples need to be spun during data collection. If we record data quickly while the sample rotates and then raster scan the beam across the sample as well then we can eventually build up a picture of where the diffraction signal comes from inside a material. Recent developments have allowed this XRD-CT approach to be extended to samples where the crystallite size is larger than the beam size and so the method can be used for both “single crystals” and “powders”. Imaging inside a sample using X-ray diffraction can give us a unique insight into the local chemical and strain states of the components in the system and how they interact. This is only possible now due to the development of 3rd generation synchrotron radiation sources together with faster detectors and new computational methods.

Selected Oral Communications

O1 ***Structure of Materials***

In situ studies of shape memory alloys during thermal / mechanical cycles

F.M. Braz Fernandes [a] , J.P. Oliveira [a], R.M.S. Martins [a,b], S.V. Correia [a], N. Schell [c]

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Keywords: Shape Memory Alloys, XRD, Synchrotron Radiation

Shape memory alloys (SMA) are a relevant class of functional materials. Their singular properties result from complex thermal / mechanical / structural interactions that require a combination of spatial and time resolution that is only achievable by the use of synchrotron radiation. An overview of the in situ studies using synchrotron radiation in the field of shape memory alloys will be presented: from in situ studies of NiTi thin film growth[1,2] (Fig. 1), to thermal / mechanical cycling[3], including, more recently, endodontic files under flexion[4] (Fig. 2) and laser welding[5] (Fig.3).

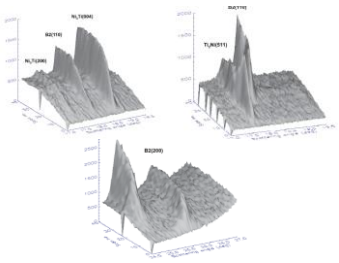

Fig. 1 - Real-time and in-situ structural design of NiTi SMA thin films.

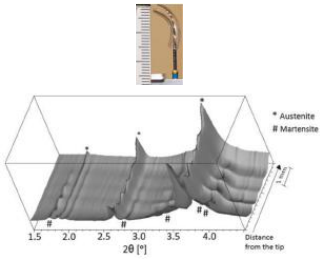

Fig. 2 - XRD Study of NiTi Endodontic Files Using Synchrotron Radiation.

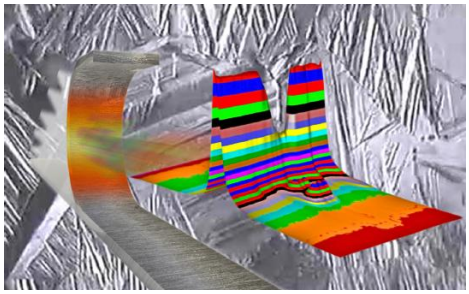

Fig. 3 - Shape memory effect of laser welded NiTi (cover photo of *Functional Materials Letters*).

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O2 ***X-ray imaging***

Unveiling the anatomy of mammal predecessors with synchrotron radiation-based micro-computed tomography: the first insights into braincase and occiput in Gorgonopsia

Ricardo Araújo[a,b,c,d], Vincent Fernandez[e], Jörg Fröbisch [b,f] & Rui M.S. Martins[a,d,g]

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Keywords: Gorgonopsia, brain endocast, braincase, occiput, osseous labyrinth

The internal structures of the braincase and occipital region in gorgonopsians so far obtained from serial grinding techniques are currently incompletely known. Gorgonopsians are key synapsid taxa for the understanding of the early steps of pre-mammalian evolution. A specimen of *Aloposaurus gracilis* (GPIT/RE/7124) collected from the upper Permian of South Africa was subjected to propagation phase-contrast X-ray synchrotron microtomography. The gorgonopsian braincase is particularly complex, especially in older individuals where extensive co-ossification and fusion occurred. Notably though, GPIT/RE/7124 is a juvenile specimen in which the sutures are clearly visible. Tomography data revealed the complex anatomy of braincase and occiput elements, as well as of the osseous labyrinth, cranial nerves and vasculature and brain endocast.

The cerebellum is broader than the forebrain, resembling the condition of other non-mammalian therapsids. The floccular complex lobes are solely delimited by the supraoccipital, yet there is an embayment on the dorsal portion of the prootics forming a lateral inflation of the cerebellum. The hypophysis is divided ventrally into two laterally-positioned pituitary lobes that communicate with the single median internal carotid foramen anteriorly.

The paths of some cranial nerves and vasculature could be discerned from the tomographies. The trigeminal nerve and the vena capitis medialis exit the brain endocast from between the pila antotica and the anterodorsal process. The vidian canal runs along the laterodorsal side of the parabasisphenoid. The internal carotids pierce the parabasishenoid lateraly and join in the median plan of the skull to exit anterior to the sella turcica. The osseous labyrinth is well preserved, however, the horizontal semicircular canal is not delimited by bone. The anterior and posterior semicircular canals are housed in the prootic, supraoccipital and opsithotic. The anterior semicircular canal is significantly larger than the posterior semicircular canal. The nopsiagonopsian brain endocast retains many “reptilian” features, demonstrating its conservative anatomy in non-mammalian therapsids.

O3 ***Structure of Materials***

Strain dependent morphological study in elastomeric nanodirelectrics

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Keywords: nanocomposites, electrical conductivity, strain, morphology

Nanodielectrics are essentially nanocomposites 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 is a network of such particles within polymer matrix. Carbon nanotubes and carbon black particles are used to form a percolative network such that the samples behave as a bulk conductor. If the material system is subjected to strain then some of the conductive pathways may be disrupted and new pathways may form. Clearly the measurement of conductivity during deformation may reveal useful data about the sample. We have established an experimental set-up to facilitate such measurements (Fig.1). This equipment can be mounted on a synchrotron beam line to enable small-angle x-ray scattering measurements to be made during the deformation cycle. As a consequence the SAXS data provides information on the morphology of the nanoparticulate structure. Combining this with the electrical properties provides us with a unique insight in to the nanostructure of these novel materials. We have performed such measurements using the NCD beamline at the ALBA synchrotron facility in Barcelona, Spain. We have prepared polyurethane based nanocomposites containing carbon nanotubes as well as silver nano wires. This poster describes the methodology of these novel experiments and we use the data to develop a model of the behaviour of these elastomeric direlectrics. This builds on previous work on the electrical properties of conductive elastomers [1].

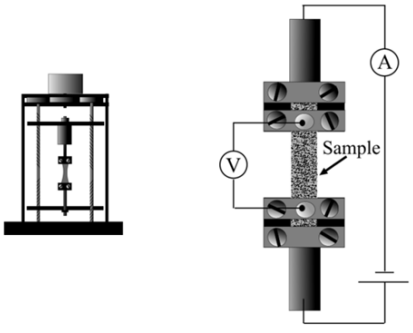

Figure 1: Set-up used to study uniaxial strain coupled with electrical conductivity measurent at Alba synchrotron.

References

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O4 ***Structural Biology***

The active-site pocket of trypsin: a dance hall

Hugo D. Correia[a], Marino F. Santos[a], Cátia S. Silva[a], Ana R. Oliveira[a], João Costa Pessoa[b], John Spencer[c], Helen Philippou[d] & **Teresa Santos-Silva**[a]

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Em homenagem a Hugo D. Correia

Keywords: urokinase-type plasminogen activator, serine proteases, protein-ligand interactions

Proteases hydrolize peptide bonds and are found in all types of organisms and cells. Among the different groups, serine proteases have been a target of excellence to medicinal-chemists since these enzymes are involved in digestion, apoptosis and different signal transduction pathways (eg blood coagulation).

Urokinase-type plasminogen activator (uPA) is a trypsin-like serine protease that binds extracellularly to the urokinase-type plasminogen activator receptor (uPAR), catalyzing the cleavage of the zymogen plasminogen to the aggressive protease plasmin.[1] It is known to be involved in a wide range of processes such as vascular disease, tumor growth, cell migration, metastasis, angiogenesis and tissue remodeling, either directly or through the activation of plasminogen. The specific inhibition of this enzyme will contribute to the treatment of diseases such as cancer, multiple sclerosis, arthritis and wound healing.[2].

In this work we use X-ray crystallography for characterizing, at the atomic level, the interaction of uPA inhibitors with bovine trypsin.

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Poster Abstracts

P1 ***Chemistry***

Nalidixic acid bio-inspired metal organic frameworks

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Keywords: BioMOFS, Nalidixic acid, ESRF, drug delivery, supramolecular chemistry

Nanoporours materials have attracted the interest of both academia and industry in various applications, as gas storage, gas separation and shape/size selective catalysis. Recently they started to be further used in drug storage and delivery as well as in medical imaging and sensing. One of the most important challenges in drug delivery research is the efficient transport and release of drugs in the body using nontoxic nanocarriers to improve their activity and metalorganic frameworks (MOFs) present the potential characteristics to solve this problem: high pore volume, regular porosity and the presence of tuneable organic groups within the framework, which allow the modulation of the structure of the framework as well as of the pore size.

From a series of different active pharmaceutical ingredients (API) tested, results with nalidixic acid have shown to be promising. Nalidixic acid is a quinolone antibiotic used for the treatment of urinary tract infections, which can also act as bacteriostatic and as bactericidal. Bio-inspired networks of this API with safe metals are being successfully explored. Coordination with different metals, including Zn, Mn and Mg, yielded novel coordination networks. The use of second ligands, such as oxalic and citric acids, has shown positive results and it represents a pathway to obtain structures with higher porosity. However, for some of these forms the structural characterization has been precluded due to the impossibility of growing good quality single crystals and therefore the full accomplishment of this work strongly relies on the acquisition of high resolution powder diffraction data to clarify the structure of all the compounds.

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P2 ***Structural Biology***

Structural insights into substrate selectivity of *E. coli* nitric oxide reductase flavodiiron protein

Patrícia T. Borges, C. V. Romão, V. L. Gonçalves, J. B. Vicente, M. Teixeira & C. Frazão

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Keywords: Flavorubredoxin, oxidoreductase, *E.coli*, nitric oxide detoxification, electron transport.

Flavodiiron proteins (FDPs) are part of the microbial response machinery for oxygen and/or nitric oxide detoxification [1, 2]. FDPs crystal structures revealed that each monomer includes at least two structural domains, an N-terminal metallo-β-lactamase and a C-terminal flavodoxin like domains [3]. The first domain contains a diiron center, in which the reduction of O_2 (to H_2O) and/or NO (to N_2O) occurs [2].

E. coli flavorubredoxin (EcFlRD) is a nitric oxide reductase (NOR) that performs protection against nitrosative stress under anaerobic growth conditions [4-6]. However, while some FDPs are exclusively reactive toward NO, others react preferably with O_2, whereas others catalyze the reduction of both gases, though with different efficiencies [2, 6, 7].

In order to unveil the structural determinants that define FDPs substrates selectivity, a comparison of crystal and model structures from FDPs with different substrate affinities was performed, which included the O_2-selective FDP from *Entamoeba histolytica* (EhFDP) and the NO-reducing EcFlRD. Differences were observed at two positions within the diiron second coordination sphere: a lysine (K53) and a tyrosine (Y271) present in EhFDP, are replaced by an aspartate (D52) and a serine (S262) in EcFlRD, respectively. The kinetic and thermodynamic properties of single and double mutants of EhFDP showed that these mutants exhibited a decrease in their affinity to O_2 in contrast to an increased reactivity towards NO, when compared with the wild-type protein. For the first time, there were evidences at molecular detail on the determination of the specificity for substrates (O_2 vs NO) in the FDP family [8].

On the other hand, in order to try to convert the NO reductase *E.coli* into an O_2 reductase, its D52K, S262Y and D52K/S262Y mutations were produced, and their crystallization and crystal structures determination pursued. Hereby, we report 2.0-2.5 Å resolution 3D structures of these single and double mutants, in order to elucidate the molecular mechanism behind FDPs substrate specificity

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P3 ***Structural Biology***

Structural studies on boronic acid derivatives as uPA inhibitors

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[b]*Department of Chemistry, School of Life Sciences, University of Sussex, Falmer, United Kingdom*

[c]*Division of Cardiovascular and Diabetes Research, Multidisciplinary Cardiovascular Research Centre and Leeds Institute for Genetics Health and Therapeutics, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom*

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Keywords: urokinase-type plasminogen activator, uPA, trypsin, inhibitors, boronic acids

Urokinase-type plasminogen activator (uPA) is a trypsin-like serine protease is a human enzyme that binds extracellularly to the urokinase-type plasminogen activator receptor (uPAR), catalyzing the cleavage of the zymogen plasminogen to the aggressive protease plasmin.[1] It is known to be involved in a wide range of processes such as vascular disease, tumor growth, cell migration, metastasis, angiogenesis and tissue remodeling, either directly or through the activation of plasminogen. Therefore we are interested in the specific inhibition of this enzyme due to its role in diseases such as cancer, multiple sclerosis, arthritis and wound healing.[2] On the other hand, other serine proteases have very similar active sites, thus developing uPA-specific drugs can be challenging.

Serine proteases are the most common among proteases and are found in all types of organisms and cells. Their active site is composed by a catalytic triad of the conserved residues Ser195, His57 and Asp102.[3] These residues are critical for the charge-relay mechanism responsible for the hydrolysis of proteins. The substrate specificity is conferred by the neighboring residues of the S1 pocket where it binds. In the case of trypsin-like proteases, Asp189 is of utmost importance for enzyme specificity towards arginines and lysines.

In this work we aim to design specific inhibitors for human uPA by studying two different systems, namely human uPA itself and bovine trypsin. The compounds have been synthesized from carbamimidothioic acid (4-boronophenyl) methyl ester hydrobromide, which is a known uPA inhibitor. High-resolution X-ray crystallography structures have been determined for several ligand-trypsin complexes. Kinetic assays using bovine trypsin have also been performed. Human uPA has been cloned and is currently being overexpressed in *Pichia pastoris*.

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P4 ***Structural Biology***

The Tungstate ABC transporter – structure and function of the periplasmic TupA.

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Keywords: Tungstate; Metal ABC transporter; Protein-ligand interaction; X-ray crystallography.

Molybdenum and tungsten are essential elements widespread in living organism. Both metals are important to sulfate reducing bacterium for the biosynthesis of enzymes present in several metabolic pathways and in the catalysis of important reactions of the biogeochemical cycles. ABC-transporters, distributed in all three kingdoms of living organisms, play an important physiological role in the transport of this molecules through biological membrane structures. Tup/Mod/Wtp ABC are proposed to be the first selection gate from which the cell differentiate between molybdate, tungstate and other similar oxoanions[1]. In this work we structurally and biochemically characterized the periplasmic component TupA from *Desulfovibrio alaskensis G20*.

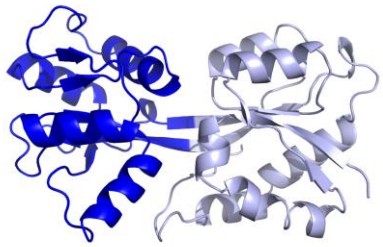

Figure 1 – Crystal structure of the *DaG20* TupA.

Using synchrotron radiation, a complete dataset was collected at ID23-1 beamline of the European Synchrotron Radiation Facility (ESRF, Grenoble). The crystals diffracted up to 1.4 Å resolution, belong to $P2_1$ space group and the structure is now in the final stages of refinement (Fig. 1). Small angle X-ray Scattering (SAXS) studies were also performed at BM29 beamline in ESRF. Data on TupA in the presence and absence of tungstate and molybdate has been collected in order to assess the putative conformational changes that occur in the periplasmic protein upon ligand interaction. The structural information was complemented with binding assays, showing that TupA binds in a 1:1 stoichiometry the molybdate and tungstate anions but has much higher affinity to tungstate than to molybdate (~10^3-times lower KD value for tungstate anions)[2]. Moreover, site-directed mutagenesis was performed, and three variants (R138K, R138Q and R138E) were produced in order to understand the specificity of TupA. Through isothermal titration calorimetry and gel shift assays it was possible to confirm that R138 is crucial to metal selectivity contributing to the comprehension of the binding mechanism of this protein.

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P5 ***Structural Biology***

Cellulosome assembly: A combined approach to characterize cohesin-dockerin interactions

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Keywords: *Clostridium thermocellum*, *Ruminococcus flavefaciens* FD1, cellulosome, cohesin-dockerin, protein microarray technology, X-Ray crystallography

Bacterial cellulosomes are complex molecular machines, specialized in the degradation of plant cell wall recalcitrant polysaccharides. The assembly and catalytic synergy of these extracellular multi-enzyme complexes rely on protein:protein interactions between two key players – the cohesins and dockerins [1-3]. Each Carbohydrate Active enZYme (CAZYme) of the cellulosome has a non-catalytic module, the dockerin, which specifically interacts with one of the several cohesins that are part of a macromolecular structure termed the *scaffoldin* [4,5]. The sequencing of bacterial genomes has revealed the presence of novel cellulosomes with diverse cohesin and dockerin modules yet to be explored.

Here, we describe a novel approach that combines a tailor-made protein microarray platform with X-ray crystallography, to identify and characterize new cohesin:dockerin interactions. The platform was developed with parallel cloning, expression and purification of >100 proteins from the cellulolytic microorganisms *Clostridium thermocellum* and *Ruminococcus flavefaciens* FD1. For microarray construction, 24 different cohesins were robotically printed onto a nitrocellulose-coated glass chip, using only minute amounts of protein. For obtaining the crystal structures of the cohesin-dockerin complexes, selected pairs have been cloned in the same vector for their simultaneous expression and purification.

The successful microarray screening of different microorganism-specific cohesin-dockerin pairs with different binding specificities and their structural characterization using X-ray crystallography shows that this integrative approach constitutes a fast and valuable means of shedding light on the architecture and dynamics of different cellulosomes.

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P6 ***Structural Biology***

Ligand discovery and structural-functional analysis of proteins involved in plant cell wall biodegradation

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Keywords: Carbohydrate microarray technology, crystallization, ITC, affinity electrophoresis, CBM, binding affinity, LysM domain.

The plant cell wall is constituted by recalcitrant polysaccharides with diverse sequences that comprise an abundant source of terrestrial biomass[1]. To efficiently degrade plant cell wall polysaccharides some cellulolytic bacterial organisms, such as *Clostridium thermocellum* and *Ruminococcus flavefaciens* FD-1, have an extracellular multi-enzyme complex with catalytic and non-catalytic carbohydrate-binding modules (CBMs)[2]. CBMs play a crucial role in enhancing the catalytic efficiency of the enzymes by eagerly targeting the substrate. The Carbohydrate Active enZYmes database (CAZY) organizes the identified CBMs by sequence similarity into different families[3]. Deposition of CBM sequences in the CAZY database is continually growing for which characterization and structure-function analysis is required.

In this study we aim to characterize the carbohydrate ligand specificities of *C. thermocellum* and *R. flavefaciens FD-1* CBMs assigned to different families in the CAZY database. We performed carbohydrate microarray screening analysis for ligand discovery[4] and crystallization screenings aiming to solve the 3D structures of the CBM-ligand complexes by X-ray crystallography[5].To complement the information provided by these methodologies we also performed ITC (Isothermal Titration Calorimetry) assays and affinity gel electrophoresis. With the implementation of this approach it was possible to elucidate ligand binding affinities of biotechnologically relevant CBMs. Preliminary results for CBMs from *C. thermocellum* family 50 LysM domain (CtCBM50) and from *R. flavefaciens FD-1* family 62 (RfCBM62) have been recently obtained and will be presented.

Financial support: *UID/Multi/04378/2013, POCI-01-0145-FEDER-007728, PTDC/BBB-BEP/0869/2014, RECI/BBB-BEP/0124/2012; SFRH/BD/100569/2014*; SFRH/BPD/68563/2010.

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P7 ***Structure of Materials***

In-situ mechanical behaviour functionally graded orthodontic NiTi wires

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Recently the use of orthodontic wires with functional graded characteristics has attracted the attention of dentists and materials engineers. This functional gradient allows for better distribution of the forces exerted in the patient mouth, which can reduce significantly the treatment time of the patients.

In this work, localized heat treatments were performed on conventional orthodontic NiTi wires in order to obtain different transformation characteristic.

The superelastic behaviour of these processes wires was analysed using in-situ X-ray diffraction analysis with synchrotron radiation, aiming at understanding the effect on the superelastic response due to the presence of different phases in the material.

These results will be discussed on the basis of their clinical consequences.

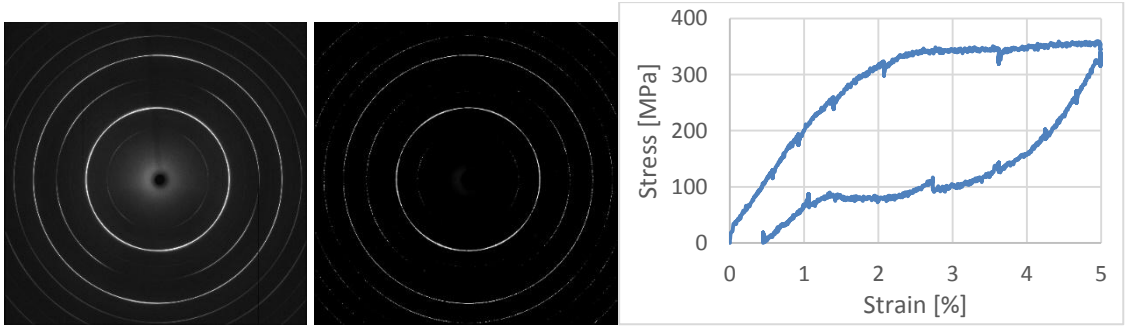

Figure 1 – Left: Debbie-Scherrer rings from the non-treated orthodontic NiTi wire; middle: Debbie-Scherrer rings from the heat treated orthodontic NiTi wire; mechanical cycling behaviour of the processed orthodontic NiTi wire.

P8 ***Chemistry***

Cocrystals of Flurbiprofen and Ibuprofen

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Keywords: Cocrystals, flurbiprofen, ibuprofen, XRPD

Flurbiprofen (FBP) (Fig. 1) and Ibuprofen (IBP) (Fig. 2) are nonsteroidal anti-inflammatory drugs (NSAID) with antipyretic and analgesic activities. Both show very low water solubility, 8 mg/L and 21 mg/L[1], respectively.

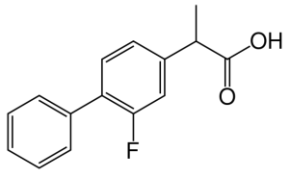

Figure 1 – Structure of Flurbiprofen.

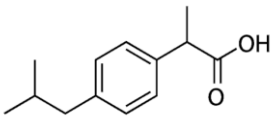

Figure 2 - Structure of Ibuprofen.

The most common strategy to alter physical and chemical properties of Active Pharmaceutical Ingredients (APIs) is the search for multicomponent crystal forms, mainly salts and cocrystals. Zwitterionic compounds favor formation of hydrogen bonds and, therefore, cocrystals. The nature of aminoacids (ionic or zwitterionic depending on the pH) makes them candidates to obtain both API salts and cocrystals. Proline shows one of the largest zwitterionic pH ranges (1.80-10.63)[2] so it was chosen for the synthesis of new FBP cocrystals using Liquid Assisted Grinding.

Reactions between FBP and D- or L-proline resulted in cocrystals with an increased solubility when compared with the API. However, some of the systems studied were impossible to crystallize and, therefore, their crystal structures could not be obtained by single crystal X-ray diffraction. So it was decided to use powder diffraction with synchrotron radiation in an attempt to solve these structures.

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P9 ***Structural Biology***

Identification of deleterious Single Nucleotide Polymorphisms (SNPs) for Human aldehyde Oxidase (hAOX): an *in silico* approach

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Human Aldehyde oxidase (hAOX) is a complex molybdo-flavo enzyme which belongs to Xantine Oxidase (XO) family (Coelho, 2015; Terao, 2016). Along with the Cytochrome P450 system (CYP450), hAOX is the major enzyme involved in the metabolism of drugs and Xenobiotics. As on 14-04-2016, 769 SNPs data are available for hAOX in the NCBI – dbSNP database. Out of 769 SNPs, 526 are denoted as non-synonymous SNPs which were subjected to eight in silico tools (I-Mutant, PolyPhen 2.0, nsSNPAnalyzer, PhD-SNP, Panter, SNP&GO, Proven and SIFT) to understand the functional consequences. All eight programs commonly suggested 122 SNPs as putatively deleterious variants and 57 as putatively neutral variants. Though 122 belong to putative deleterious category, only 65 SNPs contain validation information in the database. On-going work is directed towards the molecular dynamics (MD) simulation studies on native, inhibitor, substrate bounded structure and in SNP "G1269R" for understanding of the enzyme reaction mechanism. The present computational studies provide the impact on the functional effects (Benign or deleterious) of hAOX SNPs reported in the NCBI – dbSNP database. Existing literature (Hartmann, 2012; Foti, 2016) revealed that mutation of each amino acid residues has a variable effect on the ability of hAOX to metabolize selected substrates. Moreover, the functionally inactive variants as well as their products will have different catalytic properties. Hence, the identification of deleterious SNPs could be used for future drug design and discovery process.

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P10 ***Structure of Materials***

Strain dependent morphological study in elastomeric nanocomposites

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Keywords: Nanocomposites, electrical conductivity, strain, morphology

Nanocomposites are essentially nanodielectrics 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 and carbon black particles are used to form a percolative network in thermoset polyurethane matrix such that the sample behaves as a bulk conductor. If the material system is subjected to strain then some of the conductive pathways may be disrupted and new pathways may form. Clearly the measurement of conductivity during deformation may reveal useful information that can help to design new materials for flexible smart electronics. We have established an experimental set-up to facilitate such measurements (Fig.1). The equipment can be mounted on a synchrotron beam line to enable small-angle x-ray scattering measurements to be made during the deformation cycle. The synchronised SAXS data provide information about the morphological variations under uniaxial strain and consequent effect on electrical conductivity of nanocomposites. Moreover, one can probe the structure and dynamic behaviour of nanostructure in real time in such type of novel experiments. We have performed the measurements using the NCD beamline at the ALBA synchrotron facility in Barcelona, Spain. Nanocomposite samples prepared and studied using polyurethane containing carbon nanotubes and carbon black as filler particles. The poster describes the methodology of these novel experiments and we use the data to develop a model of the behaviour of these elastomeric direlectrics. This builds on previous work on the electrical properties of conductive elastomers [1].

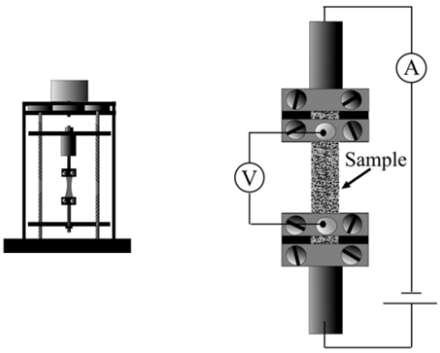

Figure 1: Set-up used to study uniaxial strain coupled with electrical conductivity measurent at Alba synchrotron.

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P11 ***Molecular Physics***

Comparison Between Electron Energy Loss Spectrum and VUV Photoabsorption Spectrum of Halothane.

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Keywords: Synchrotron radiation, VUV photoabsorption, Electron Energy Loss, Anaesthetics.

Halothane ($CF_3CHBrCl$) was widely used in the past as an halogenated anaesthetic in medicine and although there are not precise data on the amount used, an estimate of 10 kilotons/year [1], where 80% of the gas used is exhaled by the patient unchanged was reported [2]. The interaction of halothane with UV radiation has raised environmental concerns since it is delivered to the Earth lower atmospheric layers and has an estimated lifetime of about 7 years, which allows it to reach the troposphere and the lower layers of the stratosphere [1]. Reaching these altitudes where the solar actinic flux is high and UV radiation of lower wavelengths is present, photolysis may occur leading in particular to chlorine and bromide release [3]. Such may certainly have serious consequences regarding the ozone layer integrity.

Under dipolar conditions (in which the incident electron energy is relatively high and the scattering angle is small) there is almost no appreciable momentum transferred to the target molecule and the electron energy loss spectra is comparable to the VUV photoabsorption spectra.

In this study we report on the pseudo-optical spectrum of electron collisions with halothane in dipolar conditions and it is compared against the VUV photoabsorption spectrum of halothane [3]. Electron-halothane collisions were performed in the High Resolution Electron Energy Loss Spectrometer (HREELS) in the Lisbon laboratory, VG-SEELS 400 [4]. The VUV photoabsorption spectrum was recorded at the UV1 beam line of the ASTRID synchrotron facility, ISA at the Aarhus University, Denmark [5].

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P12 ***Structural Biology***

Crystal structure of GatD from *Staphylococcus aureus* suggests a structurally unexplored class of glutamine amidotransferases

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Keywords: Antimicrobial resistance, Peptidoglycan biosynthesis, Glutamine amidotransferase, X-ray Crystallography

Peptidoglycan biosynthesis relies in complex machinery where several enzymes are involved in a multitude of sequential synthetic steps. Specifically at the peptidoglycan amidation stage, the bi-complex of enzymes MurT-GatD was recently identified [1]. GatD is a glutamine amidotransferase (GAT) responsible for ammonia transfer from free glutamine to MurT which will further amidate the peptidoglycan precursor lipid II [2]. In this work we report the crystal structure of *Staphylococcus aureus* GatD at 1.9 Å resolution [3]. The active site comprises residues C94 and H189, highly conserved in all class I GATs. However, the glutamate residue that forms the catalytic triad in this class of enzymes is absent in *Sa*GatD, indicating the presence of a catalytic dyad, instead. Interestingly, a free glutamine amino acid is found at the surface of the protein, close to the active site tunnel and establishing hydrogen bonds with R128, suggesting that this arginine might be important for attracting the substrate towards the catalytic site. The particular catalytic site, in combination with other structural determinants of *Sa*GatD distinguishes it from the most well-known GAT enzymes, possibly representing a newly unexplored GAT family. The knowledge derived from this work is determinant to understand how GatD act as glutamine amidotransferase in *S. aureus* peptidoglycan amidation process. Characterization of key enzymes for *S. aureus* as potential drug targets is urgently needed to face the current multi-resistance bacteria crisis in healthcare.

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P13 ***Structure of Materials***

Ni-Ti surface with depressed nickel concentration prepared by plasma immersion ion implantation

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Keywords: Ni-Ti shape memory alloy, plasma immersion ion implantation, biocompatibility

Ni-Ti is commonly used in biomedical applications. The shape memory effect and superelasticity of Ni-Ti assure the recovery of the original shape even after large deformations and the maintenance of a constant applied force in correspondence to significant displacements. Yet, the wide spectrum of applications in implantology imposes special requirements on the biocompatibility of Ni-Ti.

The alloy (≈ 50.4 at. % Ni) selected for this study is austenitic (superelastic) at body temperature. In the frame of the AIM-74 and SPIRIT-77 projects, plasma immersion ion implantation (PIII) has been employed to modify and improve the superficial region of the alloy. The formation of titanium oxynitride (TiN_xO_y) was achieved by ion implantation of nitrogen. A Ti-rich oxide layer was obtained during the experiments carried out with oxygen. Thus, the parameters to obtain a Ni-depleted surface, which serves as a barrier to out-diffusion of Ni ions from the bulk material, have been successfully established. The high value of film resistance (measured by electrochemical impedance spectroscopy) suggests a very good corrosion resistance, which can be associated with the low Ni concentration at the surface of film. Furthermore, nanostructured Ni-Ti surfaces have been produced.

Synchrotron radiation-based X-ray diffraction data acquired in transmission mode show that the PIII technique only changes the structure of the Ni-Ti alloy top layer preserving superelastic behaviour at body temperature (PIII experiments carried out without intentional heating of the substrate holder). Techniques like thermal oxidation and nitriding also lead to an improved corrosion resistance and Ni-depleted Ni-Ti surface but require high processing temperatures leading to modification of the phase transformation characteristics and loss of specific mechanical properties.

P14 ***Structure of Materials***

In-situ observation of Ni-Ti thin film growth by synchrotron x-ray scattering: self-shadowing and surface diffusion effects

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Keywords: Ni-Ti films, shape memory alloys, co-sputtering deposition

A real understanding of the underlying growth mechanisms and microstructural development of Ni-Ti shape memory alloy films requires sophisticated *in-situ* techniques. *In-situ* studies of Ni-Ti film growth were performed at the Rossendorf Beamline (ROBL) at the European Synchrotron Radiation Facility (ESRF), using a sputtering deposition chamber inserted into the six-circle diffractometer.

Polycrystalline Ni-Ti films were deposited at ≈ 470°C onto TiN buffer layers (thickness of ≈ 15 nm) previously grown onto thermally oxidized Si(100) substrates. For Ni-Ti films deposited without applying substrate bias voltage V_b, the 110 diffraction peak of the B2 phase of Ni-Ti (in Bragg-Brentano geometry) dominated at the beginning of the deposition, while the B2(211) diffraction peak assumed higher values at a later stage of the deposition. It is suggested that the thin TiN buffer layer exhibits a rough or granular surface with different crystal facets playing an important role on the growth direction of the columnar crystals of Ni-Ti, together with the fact that the targets are tilted 30° away from the substrate normal. During the initial Ni-Ti deposition period, there is a (110) stacking and a columnar growth such that <110> lies close to the substrate normal because (110) is the more densely packed crystallographic plane for the B2 structure. However, due to the surface morphology of the TiN layer and the geometrical shadowing effects, as growth proceeds the growth direction is more influenced by the direction of incident particles. This favoured the diffraction peak of (211) of the B2 phase. Therefore, it is suggested that this crystallographic preferential orientation development is associated with the combined effects of low surface mobility and shadowing. In the case of the Ni-Ti film deposited with V_b the <110> oriented grains dominated since the beginning. The increase of the energy of the ions bombarding the film surface during growth results in an enhanced mobility of the ad-atoms in the near-surface region. Thermodynamics drives the system towards the minimum possible sum of surface and strain energies under the restrictions imposed by kinetics. The increase in the energy of the ad-atoms allows them to diffuse on the substrate surface.

P15 ***Structure of Materials***

Understanding the development of morphology during additive manufacturing of scaffolds for tissue engineering using time-resolving small angle x-ray scattering

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Keywords: Tissue Engineering, Regenerative Medicine, Additive Manufacturing, scaffolds

Tissue engineering is an emerging technology which applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue function. Developments in cell biology and bioengineering provide a realistic capability to generate tissue in the laboratory using an engineered extracellular matrix or scaffolds and biologically active molecules. At CDRsp we have focused on the engineering required to produce scaffolds with a define architecture using a bioextruder technique developed at CDRsp. The scaffold plays an important role in the tissue evolution as it mimics the natural extracellular matrix in particular promoting cell adhesion, proliferation, differentiation and new tissue formation. We know that the mechanical properties of the scaffold are critical in these functions as well as the shape and size of the environment for the implanted cells.

The processing of the biocompatible polymer in to the scaffold is performed in the melt phase of the polymer, which allows the shape to be defined, and that shape is retained as the molten polymer transforms to a solid, in many cases through crystallisation. It is well known in polymer science that the method used to process and shape the material in to the useful end object plays an important role in the definition of the final properties. The additive manufacturing process employed in this work is no different. We have small angle x-ray scattering techniques to probe the structure and morphology of the scaffolds produced. Small changes to the processing parameters has a significant effect on the crystal morphology present in the scaffold. We can also influence the structure and morphology through the addition of small quantities of nanoparticles such as graphene nanoflakes which also impact on the cell adhesion, proliferation, differentiation. When implanted scaffolds with tissue will experience stresses and for example in the case of bone regeneration these stressed may be quite high as the scaffold acts as a load bearing element. We have used *in-situ* time-resolved small-angle x-ray scattering on the NCD ALBA beamline to explore the effect of deformation on the structure and morphology of the scaffold. The results obtained in these works allow us to optimise the design of the scaffolds for utilisation to support tissue formation and subsequent implantion.

P16 ***Structure of Materials***

In-situ mechanical behaviour of superelastic NiTi laser welded joints

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Keywords: In-situ analysis, synchrotron radiation, laser welding, NiTi shape memory alloys

Preserving the functional properties, superelasticity and shape memory effect, after joining processes involving NiTi shape memory alloys is fundamental for its potential applications. Previously it was observed that these laser welded joints were able to sustain a high number of cycles (600) up to 10% strain with no fracture.

The microstructural changes induced by the laser welding procedure may have some influence on the peculiar behaviour exhibited by the joints. To fine probe the base material and thermally affected regions, synchrotron X-ray diffraction was used while performing load/unload solicitations. The aim of this work is to emphasize the role of different microstructural constituents on the superelastic cycling of the laser welded joints.

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P17 ***Structural Biology***

Development of a diagnostic tool for Chronic Myeloid Leukemia: a structural perspective

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Keywords: Chronic myeloid leukemia, diagnostic methods, small angle x-ray scattering.

Chronic myeloid leukemia (CML) affects 2/100000 adults per year, representing 15% of all types of leukemia in adults (data from IPOFG). CML is characterized by a reciprocal translocation between chromosomes 9 and 22, which creates the fusion gene BCR-ABL. Breakpoint locations are usually between exons e13 and e14. These breakpoints in the ABL gene are also variable and the transcribed mRNA has either an e13a2 (b2a2) or an e14a2 (b3a2) junction. The e13a2 and e14a2 BCR-ABL transcripts differ in length by 75 bp, encoding constitutively an active kinase, which is central to the pathogenesis of the disease [1]. In 95% of the patients with CML the e13 and e14 fusion variants are present [2].

The aim of this project is to structurally characterize the different components of a biosensor for CML diagnostic developed by Prof. Pedro Baptista group, using small angle x-ray scattering (SAXS). By SAXS, we characterized the biological components of the biosensor separately: hairpin(A), target sequence(B) and revelator(C)) and also the interaction between them (AB and ABC) – Fig. 1. Several datasets were collected in the beamline BM29 at the European Synchrotron Radiation Facility (ESRF, France) and EMBL P12 at PETRA III (Deutsches Elektronen-Synchrotron, Germany).

The Rg, I_0 and D_{max} obtained are in agreement with the expected values for this type of biomolecules and the *ab initio* bead-models are consistent with the bioinformatics simulations. These results support the previous fluorescence, UV-vis spectroscopy, dynamic light scattering and zeta potential data, allowing the visualization of the behaviour of the DNA in the biosensor.

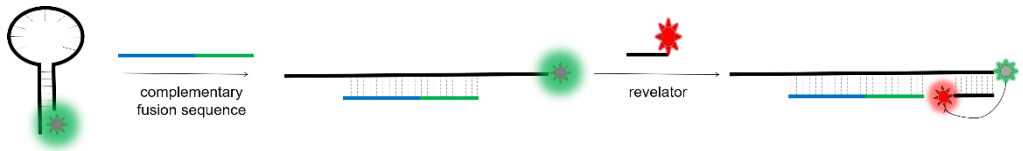

Figure 1. Schematic representation of the recognition principle used in the developed biossensor (courtesy of M. Cordeiro from Baptista's lab).

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P18 ***Structural Biology***

An Integrative Study to Reveal the Carbohydrate Specificities of the CBMomes of Two Cellulolytic Bacteria

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Keywords: CBMome, Carbohydrate-Binding Modules (CBMs), Carbohydrate Microarrays, CBM-carbohydrate specificity.

Plant cell-wall polysaccharides present enormous potential as resources for dietary, bioenergy and industrial applications. Several anaerobic microbial organisms have evolved a multi-protein complex, the Cellulosome, which efficiently contributes for plant cell wall polysaccharide biodegradation. Cellulosomes are composed of modular Carbohydrate Active enZymes (CAZymes) where the catalytic modules are appended to non-catalytic carbohydrate-binding modules (CBMs), that potentiate the enzyme's catalytic efficiencies[1]. Bacterial genome sequencing has revealed numerous putative CBM sequences, deposited in CAZy database (http://www.cazy.org), and for which specificities await elucidation. In this communication we will present results of our integrative approach combining carbohydrate microarray technology[2] with X-ray crystallography to investigate novel CBM-carbohydrate interactions and CBM-ligand specificities for two cellulolytic bacteria belonging to different ecological niches: *Ruminococcus flavefaciens* FD-1 and *Clostridium thermocellum*. All known CBMs from the genome of these bacteria (the 'CBMome') were cloned, expressed and purified using a high-throughput platform. Carbohydrate microarray analysis using plant and fungal cell wall representative sequences have shown that the two bacteria have CBMs with different carbohydrate-binding activities[3]. Studies also involved a sequence-defined gluco-oligosaccharide microarray[2], which revealed the binding specificity of a *C. thermocellum* CBM assigned to CAZy family 11 (*Ct*CBM11)[4]. The atomic information obtained by X-ray crystallography of this CBM in complex with its natural ligand, revealed the mechanism of carbohydrate recognition. These integrative studies are important to elucidate the cellulolytic capabilities of these bacteria at the molecular level. The results derived will provide relevant insights for biotechnological applications.

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P19 *Structure of Materials*

Microstructural Characterization of NiTi Shape Memory Alloys at First Step of Rotary Hot Forging

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Keywords: Shape Memory Alloys, Thermomechanical Process, synchrotron radiation X-ray.

NiTi alloys are very attractive due to their functional properties. However, the major challenges in producing these materials, which present both superelasticity and shape memory effect, are: the control of their composition, regarding the percentage ratio of Ni and Ti, and to reduce the impurities, such as carbon and oxygen.[1]

The production of NiTi usually involve several steps of hot or/and cold thermomechanical processing.[2] The present work presents the structural characterization of a Ni-rich NiTi alloy bar, produced in laboratory scale aiming at massive production in the future. The thermomechanical processing was performed by rotary forging and involved a step of hot work at 800ºC. Microstructural characterization of the produced materials was performed using Differential Scanning Calorimetry (DSC) [3] coupled with synchrotron radiation based X-ray diffraction. This way, it was possible to obtain information about the phases present in this step and the material deformation heterogeneity along the radial and the longitudinal direction with high spatial resolution. These results are important to optimize the next forging steps.

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P20 ***Structural Biology***

Iridium- and platinum-based CORMs – Synthesis, FTIR characterization and structural overview of protein-adducts

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Keywords: CO-Releasing Molecules (CORMs), Metal-protein complexes, Protein-ligand interaction, X-ray crystallography

Carbon monoxide, beyond its well-known toxic potential, is a signaling molecule playing an important role in several biological processes such as inflammation and apoptosis. Fifteen years ago, CORMs were proposed as promising prodrugs for the therapeutic use of CO. These molecules are able to transport and release CO in the blood and its pharmacokinetics strongly depends on the interactions established with blood proteins.[1]

Ruthenium-based CORMs have been extensively studied.[2,3] However, other metal-based CORMs are being progressively analyzed. Herein, we present a structural study of an iridium- and platinum-based CORMs (ALF_MS2 and ALF_MS3, respectively) which were synthesized and characterized by FTIR according to the available literature.[4] Both compounds were soaked with Hen Egg White Lysozyme (HEWL) and two structures diffracting up to 1.2 Å resolution were obtained. The HEWL•ALF_MS2 structure reveals an adduct next to Arg14 and His15: the unique CO moiety was kept in the adduct while the remaining ancillary ligands have been replaced by water molecules. Interestingly, the HEWL•ALF_MS3 structure also exhibits an adduct next His15 but, in addition to the unique CO, two out of the three Br^- ions were also modelled (Figure 1). For the first time, the maintenance of the ancillary ligands upon interaction with the protein was observed by X-ray crystallography.

Similarly to the Ru-based CORMs, the insights now obtained can be useful for the elucidation of the mechanism of CO release of this type of complexes. The data obtained suggests that these CORMs are stable enough to avoid an immediate release of CO upon protein interaction, increasing the life-time of CO in circulation and improving its therapeutic properties. Further studies on these subjects are required to a putative use of Ir- and Pt-based CORMs as viable bioactive agents.

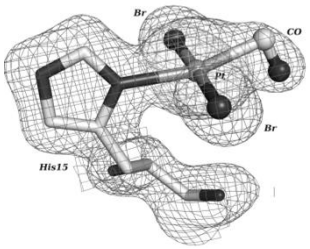

Figure 1. – Structural representation of the HEWL•ALF_MS3 adduct

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P21 ***Structure of Materials***

Nanotomography of porous membranes applied in nanofiltration

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Keywords: X-ray nano-holotomography, Mesoporous membranes

Mesoporous membranes (pore size <50 nm) have a wide range of applications, namely they are used for the filtration of nanoparticles which are potentially harmful for the environment and human health. In order to potentiate the development of novel and more efficient mesoporous materials it is essential to choose an adequate characterization technique that is capable of reaching a proper characterization in the nanoscale, namely regarding pore morphology, size, distribution, specific surface area and their interconnectivity. Amongst various tomography techniques, x-ray nano-tomography is one of the most promising [*Bandla et al., da Silva et al., Mokso et al.*].

A thin film of aluminium was deposited onto Al_2O_3 membranes (*Whatman* pore size 100-200 nm) by RF magnetron sputtering. Cylinders with 10 µm of diameter and 60 µm of height were extracted from the thin film and substrate by FIB, with the porous film positioned in the longitudinal axis, and mounted on the sample holder. Nanoscale zoom X-ray holotomography [Mokso et al.] based on a conic beam focalized by Kirkpatrick Baez multilayer mirrors was carried out at the ESRF Nano-imaging beamline (ID16A). An x-ray energy of 33.6 keV was selected to acquire radiographs with 7 and 10 nm of pixel size. A FReLoN detector (2048*2048 pixels) was used to record images at 4 predefined sample-detector distances at 1570 angular positions for each distance (total of 6280 projections). Afterwards, those projections were processed for the phase retrieval and the tomographic reconstruction.

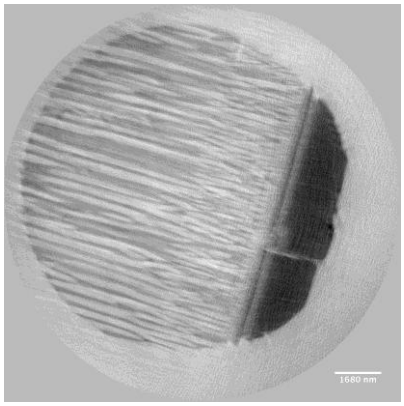

Figure 1. Example of a reconstructed slice.

Reconstructed images reveal the presence of two-level porosity – Al_2O_3 substrate (pore size 100-200nm) and Al thin film (pore size <50nm) (Fig. 1). Although further data processing and segmentation is still necessary, the preliminary evaluation of the tomographic reconstruction and phase retrieval revealed that the x-ray nano-holotomography technique can reach a resolution under 100 nm. Thus, the development of advanced algorithms for processing the data are of upmost importance in order for the technique to reach its full potential.

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P22 ***Structural Biology***

Unveiling the role of Dps from *Deinococcus radiodurans*

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Keywords: metal, DNA, X-ray fluorescence imaging, SAXS, X-ray crystallography

Deinococcus radiodurans is an extremophile bacterium, with the ability to survive to different extreme conditions such as high doses of ionizing and UV radiation, desiccation, and H_2O_2. This organism has a high intracellular ratio of manganese *versus* iron when compared to other radiation sensitive bacteria [1-2].

D. radiodurans contains two genes coding for DNA-binding proteins from starved cells: *Dr*Dps1 and *Dr*Dps2 which have been proposed to play a crucial role in manganese and iron homeostasis, but also in the protection of DNA. These proteins have a highly conserved structure as dodecameric hollow spheres [3-4], and both *Dr*Dps have the capacity to store and release iron and manganese [5].

In order to structural characterize the N-terminal tails of these proteins, which were not possible to model from the crystallographic data, we performed Small Angle X-ray Scattering (SAXS) studies in ID02 and BM29 beamlines at ESRF. The output led us to determine the position of these N-terminal tails, which are solvent accessible located outside of the dodecamer assembly.

All of our *in vitro* data is now being complement with studies in *D.radiodurans*, using X-ray fluorescence microscopy in ID16A beamline at ESRF, in order to localize intracellularly the different metals at nano-resolution. The data obtained show that manganese is concentrated in the cytoplasm, while iron is located close to the membrane.

In conclusion, we have conducted several experiments at ESRF in order to characterize our protein targets. We have started our studies by performing X-ray crystallography, followed by structural model determination in solution using SAXS, and more recently we have been using X-ray fluorescence microscopy at nano-resolution. All of these studies have been crucial to understand the function of *Dr*Dps in the radiation resistant organism *D.radiodurans* and correlate with the Mn/Fe homeostasis and ultimately with the resistance mechanisms against radiation.

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P23 ***Structural Biology***

Structure of human RuvB-Like 2 provides a mechanism for coupling between ATP binding and mechanical action.

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Keywords: RuvBL2, ATPase, DNA-binding

RuvBL1 and RuvBL2 are highly conserved ATPases known to have a key role in many cellular pathways, and cause cancer when deregulated. They use chemical energy obtained through ATP hydrolysis to exert helicase activity. To date, only the 3D structures of the full-length RuvBL1 hexamer [1], the RuvBL2ΔDII hexamer [2] and the RuvBL1ΔDII/RuvBL2ΔDII dodecameric complex [3] have been determined for the human proteins. Recently, the full-length dodecameric complex of RuvBL1/RuvBL2 from *Chaetomium thermophilum* was also determined [4, 5].

In this work, we have obtained the crystallographic structure of full-length RuvBL2 at 2.8 Å, in the apo form. By comparing our structure with the homologous structure from *C. thermophilum* and with the truncated human structure, both bound to ADP, we are able to propose a mechanism of coupling ATP hydrolysis to mechanical action. In summary, we suggest that nucleotide binding elicits a change in the neighbouring residues of the binding pocket that leads to movement of the N-terminus into direct contact with the adenine ring, thus creating an interface that causes the movement of domain 2. The high mobility of domain 2 has been predicted by molecular modelling [2], and our SAXS results support this mobility. We also analyse DNA binding of RuvBL2, supporting a mechanism whereby RuvBL2 initiates binding in the monomeric form, and forms rings upon interaction with DNA. We also show that, while hexameric RuvBL2 is unable to bind DNA, it is able to do so when in the presence of monomeric RuvBL1, suggesting that interaction with DNA may be mediated by RuvBL1.

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P24 ***Chemistry***

XANES applied to the study of tungsten speciation in molybdenites: optimisation of mineral resource efficiency through the recovery of molybdenite from mine wastes

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Keywords: XANES, Tungsten, W L_3-edge, Molybdenite, Tungstenite

It is known that in natural compounds, the capability of W^{6+} to form tungstate (like wolframite, $(Fe,Mn)WO_4$ and scheelite, $CaWO_4$) dominates compared to that of W^{4+} in forming the sulphide (WS_2). Although, these two cases were observed in Portuguese molybdenite (MoS_2) samples: tungstate in MoS_2 fragments collected at Carris and Venturinha abandoned mines and tungsten sulphide in molybdenite fragments from different veins at Borralha mine (Silva *et al.*, 2015).

The crystal structure of molybdenite is based on the stacking of [S-Mo-S] layers with Mo^{4+} cations in prismatic coordination between two superimposed closest-packed layers of S^{2-} anions, being isostructural with tungstenite (WS_2), a mineral that seldom occurs in Nature. The layered nature of these minerals enhances the possibility of intercalation of organometallic species and cations (Benavente *et al.*, 2002), making them potentially useful for new technological applications.

In the wolframite crystal structure, the W^{6+} ions are in a distorted octahedral coordination, while in scheelite W^{6+} ions have tetrahedral coordination; tungsten is linked to oxygen in both cases. In tungstenite, W^{4+} is in pyramidal coordination surrounded by sulphur ions. Therefore, the behaviour of tungsten in W-bearing molybdenite will be or the diadochic replacement of Mo or the formation of nanophases with oxygen.

The results obtained on W L_3-edge through an X-ray absorption spectroscopy (XANES) study at the European Synchrotron Radiation Facility (ESRF, Grenoble/France), beamline BM 25A (SpLine), combined with geochemical data, are described and discussed for W-rich molybdenite from various provenances. The final aim of the present study is to contribute to the optimization of the mineral resources by recovering valuable dump material from previous mining exploration activities.

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P25 ***Structural Biology***

Dps1 from *Deinococcus radiodurans*: role of transition metals in the ferroxidase center and DNA interaction

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Keywords: X-ray crystallography, radiation resistance, Mn/Fe, homeostasis, ROS.

The existence of high Mn/Fe intracellular concentration ratio in radiation-resistant bacteria but not in sensitive microorganisms supports the idea that Mn(II) accumulation, with low Fe, might be a wide-spread strategy that facilitates survival in stress conditions [1]. Small Mn(II) complexes have been shown to be able of catalytically carrying out some of the same antioxidant reactions of manganese metalloenzymes, such as superoxide dismutase and catalase, thus both postulated to play a role in radioresistance.

Deinococcus radiodurans is interesting in this context, due to its high manganese content compared with other resistant bacteria species. This Gram-positive, red pigmented bacterium is a champion of extreme resistance for its capacity to overcome oxidative stress incurred by reactive oxygen species (ROS), which are produced upon exposure to harmful conditions such as desication, starvation and radiation.

Although it is proposed that Mn/Fe ratio plays a crucial in *D.radiodurans*, knowledge on the Mn and Fe homeostasis in this bacterium is very limited. There is no gene that encodes for a typical iron-storage protein from the ferritin family but instead it contains two enconding genes for DNA-binding proteins from starved cells (Dps): *Dr*Dps1 is the product of DR2263 and *Dr*Dps2 the product of DRB0092. *Dr*Dps have been demonstrate to incorporate both iron and manganese suggesting that these proteins are involved in these metals cellular homeostasis and this may perform an effective role in the protection of *D. radiodurans* against oxidative stress. Furthermore, these proteins can physically interact with DNA adding more protection to cellular macromolecules towards ROS [2,3].

Using X-rays crystallography, we are focusing on understanding the interaction of *Dr*Dps1 with the DNA and its role in metals storage, to uncover the sites of binding of metals in the protein structure.

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P26 ***Structural Biology***

Marine Iron Scavenging: a putative siderophore interacting protein from the marine bacterium *Shewanella frigidimarina*

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Keywords: siderophore-interacting protein, *Shewanella frigidimarina*, siderophore reduction, iron scavenging

The great oxygenation event (GOE) caused a dramatic change in the bioavailability of iron. [1] The rise of oxygen in the atmosphere led to the oxidation, and precipitation of the previously readily accessible ferrous iron, Fe(II). [2] Notwithstanding its lower abundance, iron remained essential to most life forms, and in order to grow organisms have evolved diverse strategies for acquiring iron. A widely employed strategy is the extracellular release of siderophores, small molecules that have a high affinity for ferric iron, Fe(III). [2, 3]

Siderophores are extracellularly released in the apo-form and then incorporated via specific receptors as ferric complexes. [4] In order to be utilized, iron has to be released and multiples routes have been proposed for this, including: the hydrolysis of the siderophore by esterases and siderophore recycling. [5] In 2015, Kunhua Li et al. proposed that siderophore recycling is mediated by specific proteins that can be considered in two families: one is the ferric reductase family (FSR), characterized by proteins that contain an atypical 2Fe-2S cluster and the siderophore-interacting protein (SIP) family that have a flavin cofactor. [5,6]

Here, we present the recombinant production, crystallization and preliminary crystallographic and biochemical characterization of a putative SIP from the marine bacterium *Shewanella frigidimarina*.

The aim of this work is to understand the mechanistic and structural details of these families of proteins attaining a molecular insight of the iron scavenging strategies used by bacteria. This knowledge is essential for the development of new drugs that combat the virulence of pathogens, and it can also promote an efficient use of siderophores in bioremediation activities.

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P27 ***Environmental and Cultural Materials***

Yellow colouring in glazed tiles from the Pena National Palace and the National Tile Museum: understanding our cultural heritage through XANES, μ-Raman, μ-EDXRF and Optical Microscopy

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Keywords: Glazed Tiles, Yellow pigments, XANES, Raman, EDXRF

Portuguese glazed tiles are unique in artistic valour and historical indoor and outdoor widespread application. Understanding the materials used in an attempt to protect and increase the lifespan of this art form has been a driving force in the application of different analytical techniques.

In the present work an attempt has been made to ascertain the possibility of finding a common start point for the yellow colour in glazes from previously studied 17th century tile panels from the National Tile Museum and 19th century tiles from the external façades of the Pena National Palace in Sintra [1]. Non-destructive characterization techniques were used such as X-ray Fluorescence, Variable Pressure Scanning Electron Microscopy, Optical Microscopy and Raman spectroscopy [2] along with Synchrotron Radiation X-ray Absorption Spectroscopy. Prior measurements performed at ESRF beamline BM23 demonstrated the role of lead and antimony on the yellow colour as a dispersed pyrochlore-type Sb-oxide [3-4]. An attempt was now made with in house techniques to infer if the same situation was possible for the Portuguese 19th century tiles of Pena National palace. Results so far indicate some compositional similarities but also structural differences and so the access to synchrotron radiation facilities for the use of X-ray absorption techniques such as XANES is of the utmost importance to clarify Sb speciation on yellow pigments in these materials.

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P28 ***Structural Biology***

Arsenite oxidase: Bacterial Oxidation for Bioremediation

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Keywords: Arsenic, Arsenite oxidase, Molybdoenzymes, X-ray crystallography, Site-directed mutagenesis, Kinetic Enzymatic Assays.

Arsenic is broadly distributed in the environment, arising from both natural and anthropogenic processes. It is commonly found in the form of arsenite ($As^{III}O_2^-$) and arsenate ($As^{V}O_4^{3-}$), highly toxic species. Arsenite is considered to be more toxic than arsenate and can be oxidized to arsenate chemically or microbially. The arsenite oxidizing bacteria either can gain energy from arsenite oxidation or act in detoxification processes [1].

Arsenite oxidase (Aio) is a metabolic enzyme that is responsible for the oxidation of arsenite into arsenate, comprising a molybdenum coordinated by two piranopterins and several Fe-S clusters [2].

The crystal structure of Aio from the chemolithoautotrophic arsenite oxidizer *Alphaproteobacterium Rhizobium sp.NT-26* was previously determined at 2.7 Å resolution [2]. Our aim is to clarify the reaction mechanism of the enzyme by structurally characterizing native NT-26 Aio bound to substrate analogs and inhibitors as well as several mutants of the enzyme, using a combination of X-ray crystallography and kinetic assays.

Using site-directed mutagenesis different variants were prepared – F108A, Q726G, D169A/N and E453A/Q. Protein expression, purification and crystallization were carried out. The wild type protein and the first two mutants were crystallized using PEG400 as precipitating agent, diffracting up to *ca* 2.0 Å resolution. The electron density obtained clearly shows the mutated residues and the structures are in the last stages of refinement. Kinetic enzymatic assays, using the physiological electron acceptor cytochrome c as well as the artificial electron acceptor DCPIP (2,6-Dichlorophenolindophenol), are under way.

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P29 ***Structural Biology***

Towards an engineered O_2-tolerant-like proximal FeS cluster in a [NiFeSe] hydrogenase

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[NiFeSe] hydrogenases (Hases) are highly active for proton reduction and form different oxidised states from [NiFe] hydrogenases, which are rapidly reactivated under reducing conditions[1].

O_2-tolerant membrane-bound [NiFe] Hases (MBHs) are able to oxidize H_2 in presence of O_2. This is due to the presence of a special proximal FeS cluster in O_2-tolerant hydrogenases coordinated by two cysteines at positions where conserved glycines are found in the O_2-sensitive [NiFe] Hases.

Desulfovibrio vulgaris Hildenborough (*Dv*H) is an anaerobic organism that contains several different hydrogenases, including a [NiFeSe] Hase[2,3]. We developed a homologous expression system for the *Dv*H [NiFeSe] Hase and generated two variants, G20C and G126C, where the conserved glycines next to the proximal FeS cluster were mutated to cysteines, in an attempt to mimic the proximal FeS cluster of O_2-tolerant Hases. The G126C variant was successfully expressed, purified and crystalized. X-ray crystallography data at 1.4 Å was obtained at ESRF beamline ID23-2, revealing that C126 does not form a bond with the proximal [Fe_4S_4] cluster, which retained its standard cubane structure. As expected, C126 displaced one of the conserved water molecules near the proximal centre, and the degree of oxidative damage to the cluster was markedly lower (13%) than for all the other crystal structures previously obtained under similar conditions from aerobically crystallized *Dv*H [NiFeSe] Hase. This suggests that the water molecules found near the proximal cluster may play a role in its oxidative damage under aerobic conditions. Expression of G20C was more challenging, with a dramatic decrease in protein expression. Crystallization trials are underway for this variant.

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P30 ***Structural Biology***

Thiosulfate Dehydrogenases (TsdA) from *Allochromatium vinosum* and *Campylobacter jejuni*: Structural Insights into Thiosulfate Oxidation

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Keywords: **X-ray crystallography, thiosulphate dehydrogenase, *c*-type cytochrome, haem coordination, ligand switching**

The ability to perform the very simple oxidation of two molecules of thiosulphate to tetrathionate is wide spread among prokaryotes. Despite the widespread occurrence of tetrathionate formation, and its well-documented significance within the sulphur cycle, little is known about the enzymes catalysing the oxidative condensation of two thiosulphate anions. To fill this gap, the thiosulphate dehydrogenase (TsdA), enzyme from *Allochromatium vinosum*, was produced recombinantly in *E. coli* and kinetic and spectroscopically characterized [1]. The "as isolated" crystal structure of the enzyme was determined by the Single Anomalous Dispersion (SAD) method using the Fe-heme anomalous signal [2]. Moreover, we have determined the X-ray structures of TsdA in different redox states and with several ligands [3].

A. vinosum TsdA contains two typical class I *c*-type cytochrome domains with two hemes axially coordinated by H53/C96 and H164/K208 with C96 being essential for catalysis [3]. The X-ray structure showed an all-alpha structure with structural similarities to the *Rhodovulum sulfidophilum*'s SoxAX (PDB 2OZ1), and the low-redox-potential cytochrome *c*6 from *Hizikia fusiformis* (PDB 2ZBO). Interestingly, reduction of the enzyme causes a ligand switch from K208 to M209 in heme 2. Overall, our kinetic, spectroscopic and structural data lead us to propose a reaction mechanism [3].

More recently, we have also determined the crystal structure of the enzyme TsdA from *Campylobacter jejuni*. Kinetic studies with purified recombinant *C. jejuni* TsdA showed it to be a bifunctional tetrathionate reductase/thiosulphate dehydrogenase with a high affinity for tetrathionate [4]. Given the widespread distribution of TsdAs, we hypothesize that, like *C. jejuni*, many more bacteria than previously thought are capable of growth on tetrathionate. This ability might serve as an evolution advantage to quickly adapt to environmental changes and substrate availability.

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P31 ***Structural Biology***

Structural Studies of bacterial Succinate-Acetate Transporters

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Keywords: Membrane Acetate Transporters, AceTr, Satp-YaaH homologues, X-ray Crystallography

Many cells must alternate between life-styles that permit rapid growth in the presence of abundant nutrients and ones that enhance survival in the absence of those nutrients. One such change in life-style, the "acetate switch," occurs as cells deplete their environment of acetate-producing carbon sources and begin to rely on their ability to scavenge for acetate. The Acetate Uptake Transporter (AceTr) Family members play a role in this process. They are characterized for possessing six predicted transmembrane segments being found in bacteria, archaea, several fungi and protozoa (TC 2.A.96, www.tcdb.org). SatP, the only member of this family found in *Escherichia coli*, is highly specific for acetic acid and succinic acid.

We have optimized the production of homologues of SatP-YaaH from *Escherichia coli*, a 20 kDa membrane protein responsible for the acetic acid transport along with another membrane protein, the Acetate Symporter ActP [1]. A small-scale protocol was used and the production of 11 membrane targets was tested in different *E. coli* strains, growth media, induction times and growth temperature. The best targets were then selected for larger-scale purification assessing various detergents for membrane extraction and protein solubilization along with affinity and size-exclusion Chromatographies. One of the targets yielded crystals, which are currently under crystallization optimization, the best diffraction so far in a synchrotron radiation source was ~10-12 Å. No structure of AceT is yet known.

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P32 *Structure of Materials*

Using synchrotron radiation for exploring structural transformations during dissimilar joining with reactive materials

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Keywords: Joining, Multilayers, Filler, NiTi/Ti6Al4V, Synchrotron radiation

Reactive materials can be utilised to assist the diffusion bonding process of NiTi shape memory alloys to themselves and to other alloys. The reaction-assisted diffusion bonding process of NiTi /Ti-6Al-4V using either magnetron sputtered Ni/Ti nanomultilayers or Ni/Ti commercial microfoils was studied *in situ*. For this purpose, experiments were carried out at the High Energy Materials Science beamline (P07) at the German Electron Synchrotron (DESY). The oven with load capabilities at P07 beamline is ideal to follow the structural evolution during the joining process.

Ni/Ti multilayer thin films with a 2.5 μm total thickness and with 12 or 25 nm of modulation period were deposited onto the materials being joined. In alternative, up to 20 alternated thin μ-foils were placed in between NiTi and Ti-6Al-4V. Base and filler materials were placed in the oven equipped with polyimide windows transparent to the x-ray beam. Joining was promoted by applying a 10 MPa pressure and inductively heating the materials, while simultaneously x-ray scanning across the bond interface. The incident x-ray beam (0.05 x 1 mm^2) was monochromatized to 80 keV (λ = 0.01550 nm). Complete Debye diffraction rings were recorded on a 2D detector with 2048x2048 pixels. Temperature profiles up to maximum temperatures from 600 to 750 ^oC were selected.

A temperature as low as 600 ^oC is sufficient for the Ni/Ti nanomultilayer to react and release heat to achieve a sound joint between NiTi and Ti-6Al-4V. On the other hand, if thin μ-foils were used, a 650 ^oC temperature was required. The formation of undesired intermetallic phases, such as $NiTi_2$, can be minimised by decreasing bonding temperature. In situ characterization of the reaction-assisted diffusion bonding joints combined with high resolution scanning electron microscopy analysis after joining allow the most promising filler materials to be identified.

List of Communications

Invited Speakers:

IS1 ***p. 4*** Sirage, Emir ***FCT***
Portugal and LSFs in Europe

IS2 ***p. 5*** Biscari, Caterina ***ALBA***
ALBA, the Spanish synchrotron

IS3 ***p. 6*** Walsh, Martin ***DLS***
Diamond – A New Light for Life Sciences

IS4 ***p. 7*** Reichert, Harald ***ESRF***
The Next Step in the Exploitation of Storage-Ring-Based High Energy X-ray Sources

IS5 ***p. 8*** Leonard, Gordon ***ESRF***
Facilities for Structural Biology at the European Synchrotron Radiation Facility

IS6 ***p. 9*** Wright, Jonathan ***ESRF***
Looking inside materials using synchrotron X-ray diffraction

Oral Communications:

O1 ***p. 11*** Braz-Fernandes, Francisco Manuel ***Structure of Materials***
In situ studies of shape memory alloys during thermal / mechanical cycles

O2 ***p. 12*** Araújo, Ricardo ***X-ray imaging***
Unveiling the anatomy of mammal predecessors with synchrotron radiation-based micro-computed tomography: the first insights into braincase and occiput in Gorgonopsia

O3 ***p. 13*** Mitchell, Geoffrey ***Structure of Materials***
Strain dependent morphological study in elastomeric nanodirelectrics

O4 ***p. 14*** Teresa Santos-Silva ***Structural Biology***
The active-site pocket of trypsin: a dance hall

Poster Communications:

P1 ***p. 16*** André, Vânia ***Chemistry***
Nalidixic acid bio-inspired metal organic frameworks.

P2 ***p. 17*** Borges, Patrícia ***Structural Biology***
Structural insights into substrate selectivity of E. coli nitric oxide reductase flavodiiron protein

P3 ***p. 18*** Correia, Hugo ***Structural Biology***
Structural studies on boronic acid derivatives as uPA inhibitors

P4 ***p. 19*** Correia, Márcia A. S. ***Structural Biology***
The Tungstate ABC transporter – structure and function of the periplasmic TupA.

P5 ***p. 20*** Correia, Viviana G. ***Structural Biology***
Cellulosome assembly: A combined approach to characterize cohesin-dockerin interactions

P6 ***p. 21*** Costa, Raquel L. ***Structural Biology***
Ligand discovery and structural-functional analysis of proteins involved in plant cell wall biodegradation

P7 ***p. 22*** Ferreira, Fernando ***Structure of Materials***
In-situ mechanical behaviour functionally graded orthodontic NiTi wires

P8 ***p. 23*** Galego, Filipa ***Chemistry***
Cocrystals of Flurbiprofen and Ibuprofen

P9 ***p. 24*** Jayaraman, Muthukumaran ***Structural Biology***
Identification of deleterious Single Nucleotide Polymorphisms (SNPs) for Human aldehyde Oxidase (hAOX): an in silico approach

P10 ***p. 25*** Khan, Imran ***Structure of Materials***
Strain dependent morphological study in elastomeric nanocomposites

P11 ***p. 26*** Lange, Emanuele ***Molecular Physics***
Comparison Between Electron Energy Loss Spectrum and VUV Photoabsorption Spectrum of Halothane

P12 ***p. 27*** Leisico, Francisco M. P. ***Structural Biology***
Crystal structure of GatD from *Staphylococcus aureus* suggests a structurally unexplored class of glutamine amidotransferases

P13 ***p. 28*** Martins, Rui ***Structure of Materials***
Ni-Ti surface with depressed nickel concentration prepared by plasma immersion ion implantation

P14 ***p. 29*** Martins, Rui ***Structure of Materials***
In-situ observation of Ni-Ti thin film growth by synchrotron x-ray scattering: self-shadowing and surface diffusion effects

P15 ***p. 30*** Mitchell,Geoffrey ***Structure of Materials***
Understanding the development of morphology during additive manufacturing of scaffolds for tissue engineering using time-resolving small angle x-ray scattering

P16 ***p. 31*** Oliveira, Joao Pedro ***Structure of Materials***
In-situ mechanical behaviour of superelastic NiTi laser welded joints

P17 ***p. 32*** Otrelo-Cardoso, Ana Rita ***Structural Biology***
Development of a diagnostic tool for Chronic Myeloid Leukemia: a structural perspective

P18 ***p. 33*** Ribeiro, Diana ***Structural Biology***
An Integrative Study to Reveal the Carbohydrate Specificities of the CBMomes of Two Cellulolytic Bacteria

P19 ***p. 34*** Rodrigues, Patrícia ***Structure of Materials***
Microstructural Characterization of NiTi Shape Memory Alloys at First Step of Rotary Hot Forging

P20 ***p. 35*** Santos, Marino ***Structural Biology***
Iridium- and platinum-based CORMs – Synthesis, characterization and structural overview of protein-adducts

P21 ***p. 36*** Santos, Rita J. ***Structure of Materials***
Nanotomography of porous membranes applied in nanofiltration

P22 ***p. 37*** Santos, Sandra ***Structural Biology***
Unveiling the role of Dps from Deinococcus radiodurans

P23 ***p. 38*** Silva, Sara T. N. ***Structural Biology***
Structure of human RuvB-Like 2 provides a mechanism for coupling between ATP binding and mechanical action.

P24 ***p. 39*** Silva, Teresa P. ***Chemistry***
XANES applied to the study of tungsten speciation in molybdenites: optimisation of mineral resource efficiency through the recovery of molybdenite from mine wastes

P25 ***p. 40*** Silva, José Pedro ***Structural Biology***
Pinel, Denise
Dps1 from *Deinococcus radiodurans*: role of transition metals in the ferroxidase center and DNA interaction

P26 ***p. 41*** Trindade, Ines ***Structural Biology***
Marine Iron Scavenging: a putative siderophore interacting protein from the marine bacterium *Shewanella frigidimarina*

P27 ***p. 42*** Veiga, João P. ***Environmental and Cultural Materials***
Yellow colouring in glazed tiles from the Pena National Palace and the National Tile Museum: understanding our cultural heritage through XANES, micro-Raman, mmicro-EDXRF and Optical Microscopy

P28 ***p. 43*** Vieira, Marta F.M. ***Structural Biology***
Arsenite oxidase: Bacterial Oxidation for Bioremediation

P29 ***p. 44*** Zacarias, Sónia ***Structural Biology***
Towards an engineered O2-tolerant-like proximal FeS cluster in a [NiFeSe] hydrogenase

Last minute Abstract Submission

P30 ***p. 45*** Cordeiro, Maria M. ***Structural Biology***
Thiosulfate Dehydrogenases (TsdA) from *Allochromatium vinosum* and *Campylobacter jejuni*: Structural Insights into Thiosulfate Oxidation

P31 ***p. 46*** Athayde , Diogo ***Structural Biology***
Structural Studies of bacterial Succinate-Acetate Transporters

P32 ***p. 46*** Ramos, A. S. ***Structure of Materials***
Using synchrotron radiation for exploring structural transformations during dissimilar joining with reactive materials

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