# ENURS 2023

## WELCOME TO 21ST JUNE 2023

## 9TH MEETING

CENTRO EMPRESARIAL
MARINHA GRANDE

POLITÉCNICO DE LEIRIA | CDRSP CENTRE FOR RAPID AND SUSTAINABLE PRODUCT DEVELOPMENT

ENURS 2023 is the ninth edition of the annual meeting of Portuguese Synchrotron Users.The meeting will provide an opportunity to hear about the developments upgrades and new opportunities at the European Synchrotron Research Facility in Grenoble,France and at the Alba Synchrotron Light Source in Barcelona, Spain.

The meeting will also provide opportunities for users to share their recent research via oral or poster presentations.

# Organizer Committee

01 Geoffrey Mitchell

02 Rafael Tavares

03 Tiago Sieiro

04 Alana da Silva

05 Ana Rita Pedrosa

06 Ana Peixinho

07 Anabela Massano

08 Matteo Arioli

09 Fábio Gameiro

10 Liliana Gouveia

11 Margarida Filipe

12 Diana Cruz

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# PROGRAMME 21ST JUNE 2023

**10:00 Registration and coffee and refreshments**
Centro Empresarial, Zona Industrial, Marinha Grande
**11:00 Open Ceremony - Welcome and Introductions**
Professor Geoffrey Mitchell, Local Organiser
Professor Artur Mateus,
Director of the Centre for Rapid and Sustainable Product Development
Professor Pedro Amado de Assunção, Vice-President of the Polytechnic of Leiria

**11:15 Updates and Opportunities - Chair Paula Pascoal-Faria**

**European Synchrotron Research Facility**
**11:15 Michael Krisch**
Director of Research ESRF
"Extremely Brilliant Source (EBS) – a European effort to pioneer synchrotron x-ray science"
**11:45 Montserrat Soler-Lopez**
Group Head of the Structural Biology Group
"Structural Biology at the ESRF – opening up new avenues"
**12:05 Veijo Honkimaki**
Group Head of the Structure of Materials Group "Materials Science at the ESRF – towards a sustainable future"

**ALBA Synchrotron Light Source**

**12:25 Barbara Calisto,**
ALBA Synchrotron Light Source
"Opportunities at the ALBA Synchrotron Light Source"

**European Synchrotron Research Facility**

**12:55 Gary Admans,**
Business Development Officer ESRF
"Research infrastructure assistance for research in to circular materials and recycling processes"

**13:05 Q and A on morning session**

**13:15 Lunch and User Posters**

**Posters**
1. **Diogo Athayde**
"Structure-based design of neutralizing engineered protein targeting Zika virus Envelope protein
2. **Filipa Engrola**
"A biophysical and structural approach to shed light on arsenite oxidase reaction mechanism"
3. **Guilherme Vilela-Alves**
"Structural studies of bacterial PHA synthases"
4. **Alícia Candeias**
"Structural insights into host glycoprotein recognition by Bacteroides caccae: the 3D Structure of a novel member of family 32 CBM in complex with the Tn Antigen"
5. **André Gouveia**
"Exploring Deinococcus arsenic resistance as a tool for bioremediation"

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6. **João Santos**
"Impact of Metallodrugs on Cellular Membrane Lipid"

8. **Pedro Matias**
"Improving the Oxygen tolerance in a highly active [NiFeSe] Hydrogenase"

9. **Clara Martins**
"Water dynamics in healthy-to-cancer transition: Insights from Quasi-Elastic Neutron Scattering"

10. **Anabela Massano**
"Novel Biobased Composites based on Polybutylene succinate with nano-sized carbonaceous fillers"

11. **Matteo Arioli**
"Nylon 7,10: Real time X-ray diffraction and FTIR microspectroscopy studies on a novel odd-even polyamide"

12. **João Matias**
"Novel Biaxial Deformation Stage for Natural Rubber for SAXS/WAXS"

13. **Paula Faria**
"Multiscale Digital Twin of an Injection Moulding System for Plastics using the NCD-SWEET beamline at the ALBA Synchrotron Light Source"

14. **Geoffrey Mitchell**
"Bringing Injection Moulding of Plastics to the SAXS Beamline at ALBA"

15. **Daniel Silva**
"Discovering Morphology Mapping with 3D printing at the NCD-SWEET Beamline at ALBA"

16. **Daniel Silva**
"Experimental setup and equipment modelling for in-situ morphology SAXS/WAXS measurements of electrospun polymer fibres on the NCD--SWEET beamline at the ALBA Synchrotron Light Source"

**Animated Poster**

17. **Daniel Gatões**
"In-situ study of mechanical and structural behavior in metallic additive manufacturing"

**14:30 João Pedro Veiga**
ESFR Scientific Council
Chair João Matias

**14:40 Barbara Calisto**
ESUO and discussion on the formation of a ENURS Association

**15:00 Users Presentations - Chair João Matlas**

**15:00 Artur Mateus**
**CDRSP**
"Operando x-ray scattering studies of injection moulding of plastics at the ALBA synchrotron light source – towards a circular process"

**15:20 Bruno Salgueiro**
ITQB NOVA
"Structural analysis of three distinct classes of Flavodiiron proteins obtained by X-ray Crystallography and Cryo-EM Single-Particle"

**15:40 Isabel Sousa**
UA
"Interlayer intercalation of 2-mercaptobenzothiazole in layered double hydroxides grown on AA2024 alloy: an in situ study at SOLEIL synchrotron"

**16:00 Coffee-Break**

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# PROGRAMME 21ST JUNE 2023

**16:15 Users Presentations - Chair Pedro Carreira**

**16:15 Daniel Silva**
CDRSP
"Discovering morphology mapping with Operando small-angle X-ray Scattering 3D printing at ALBA"

**16:35 Andrei Salak**
CICECO
"Pressure-induced transitions in 50 mol.% Fe-to-Sc substituted BiFeO3 via in situ synchrotron X-ray diffraction study"

**16:55 Raquel Laginha**
UoC
"Advantages of FTIR Microspectroscopy with Synchrotron Radiation - an Inside View into Osteosarcoma Treatment"

**17:15 Jiajia Shen**
UNIDEMI
"Deformation behavior and strengthening effects of an eutectic AlCoCrFeNi2.1 high entropy alloy probed by in-situ synchrotron X-ray diffraction and post-mortem EBSD"

**17:35 Guilherme Vilela-Alves**
UCIBIO
"One step forward to understand the biological reduction of CO2 to Formate by Mo/W Fdhs"

**18:00 Close of Meeting**
All participants are invited to a farewell coffee.

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# ORAL PRESENTATIONS

**Michael Krisch**
The Extremely Brilliant Source (EBS) – a European effort to pioneer synchrotron X-ray science

Link:
http://enurs.ipleiria.pt/files/2023/07/ENURS_Portugal2023_MichaelKrisch_final.pdf

**Montserrat Soler-Lopez**
Structural Biology at the ESRF – opening up new avenues

Link:
http://enurs.ipleiria.pt/files/2023/07/SolerLopez_Structural-Biology_ESRF_ENURS_2023.pdf

**Barbara Calisto**
ALBA Synchrotron: Overview and opportunities

Link:
https://enurs.ipleiria.pt/files/2023/07/ALBAoverview_ENURS2023_bc.pdf

**Gary Adams**
Research infrastructure assistance for research into circular materials and recycling processes

Link:
http://enurs.ipleiria.pt/files/2023/07/GaryAdmans-ReMade@ARI-20230621-ESRF-ENURS.pdf

**João Pedro Veiga**
ESRF – The European Synchrotron

Link:
http://enurs.ipleiria.pt/files/2023/07/Pt_VH.pdf

**Barbara Calisto**
Discussion on the formation of a Portuguese Synchrotron and FEL Users Association

Link:
http://enurs.ipleiria.pt/files/2023/07/USERS_overview_ENURS2023.pdf

# *In operando* small-angle X-ray scattering studies of injection moulding of polymers

Pedro Carreira[1], Fábio A. Gameiro[1], Anabela P. Massano[1], Daniel P. da Silva[1], Marc Malfois[2], João Matias[1], Paula Pascoal-Faria[1], Artur Mateus[1] and Geoffrey Mitchell[1,*]

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**Keywords:** Injection Moulding, Digital Manufacturing, Polymers, Small-angle X-Ray Scattering.

**Abstract**: Injection moulding of polymers is currently the most common method to fabricate 3D shapes from plastics. It is a deceptively simple technique in which molten plastic is injected at high pressure into a metallic mould, the plastic cools and solidifies either by passing through a glass transition as in the case of amorphous plastics or crystallising as in the case of semi-crystalline polymers.

Now, injection moulding has developed greatly since the early days of the equipment developed by the Hyatt brothers, and in parallel plastics have also been revolutionized through careful control of the polymerization process and the development of new catalysts. At the same time, our understanding of the flow behaviour of polydisperse polymer melts and the crystallization through the emergence of new experimental procedures. The design and development of tooling for the injection moulding process is now performed digitally, moulds are evaluated using digital simulations, and fabricated using largely digital processes, although the final stages may involve manual corrections.

Injection moulding has reached this summit of technological excellence at more or less the same time that society has begun to understand some of the environmental downsides of large-scale plastic consumption. As a consequence, there is now great focus on the circular economy and the effective recycling of plastics as well as the development of new bioplastics based on renewable resources and which are biodegradable.

The process of injection moulding can be characterized by high cooling rates, intense and complex flow fields and the use of high-pressure injection using a screw extruder. Polymer processing technologies have largely developed through the use of experimental characterization post processing from which it is challenging to recover the time-evolution of the development of structure and morphology which is critical to the properties and behaviour of the final product. Injection moulding is surprisingly limited in the sensors and evaluation devices which could be used to follow such developments of structure and morphology. As a consequence, currently the design of an injected moulded product is largely limited to the definition of the shape and there is limited use of the control of the structure and morphology, the living hinge, mainly in packaging lids, is a notable exception.

This work is focused on the development of an industrially relevant injection moulding system which can be mounted on the NCD-SWEET beamline at the ALBA Synchrotron Light Source in Barcelona, Spain. This equipment enables in operando processing, providing time-resolved quantitative data on the development of the structure and morphology of the plastic within the mould cavity, during the injection moulding cycle. This presentation will briefly discuss the key aspects of the experimental system and some initial results from both synthetic semi-crystalline polymers as well as bioplastics such as polyhydroxyalkonoates and polybutylene succinate.

**Acknowledgements:** This work was financially supported by the Fundação para a Ciência e a Tecnologia FCT/MCTES (PIDDAC) through the following Projects: MIT-EXPL/TDI/0044/2021, UIDB/04044/2020; UIDP/04044/2020; Associate Laboratory ARISE LA/P/0112/2020; It is also supported by the National Agency for Innovation through the projects PAMI - ROTEIRO/0328/2013 (Nº 022158), plus EcoPlast, Materiais compósitos eco-sustentáveis para substituição dos plásticos convencionais, ref POCI-01-0247-FEDER-069002, and INNOV-AM.

# Structural analysis of three distinct classes of Flavodiiron proteins obtained by *X-ray Crystallography* and *Cryo-EM Single-Particle*

B. Salgueiro[1,*], Maria C. Martins[1], Marcos Gragera[2], Alessandro Grinzato[3], Filipe Folgosa[1], Miguel Teixeira[1], Célia V. Romão[1] and Carlos Frazão[1]

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The presence of considerable amounts of oxygen in the atmosphere forced modifications in the cellular metabolism of organisms[1]. To overcome this problem, organisms have found ways to convert both reactive oxygen (ROS) and NO (RNS) species through the synthesis of enzymes responsible for their detoxification[1]. One of the common aspects of these enzymes is the presence of Fe centers responsible for the conversion of these species to harmless molecules in organisms. Flavodiiron proteins (FDPs) are soluble enzymes, characterized with the minimal functional unit, the presence of a two-domain core, a metallo-β-lactamase-like domain, with a diiron catalytic center, followed by a flavodoxin-like domain, containing a flavin mononucleotide (FMN)[2]. Based on amino acid sequence analysis this protein family is divided into 8 classes. The aim of this study was the structural characterization of three Flavodiiron proteins (FDPs) of classes A, B and F. Class A is the simplest that harbours only the core domain: metallo-β-lactamase-like and flavodoxin-like domains. Class B, is the Class A plus a rubredoxin domain at the C-terminal, and Class F is composed by Class B domain followed by a flavo-reductase domain at the C-terminal.

Crystals of the Class A FDP from *C difficile* P28 diffracted at 2.5Å resolution collected at ALBA (Spain), and the SAXS envelope obtained from scattering data was collected at Diamond Light Source (UK). From the protein crystals of the Class B – FDP from *Escherichia coli*, we collected a 2.8Å dataset at ESRF (France). The 3D structure of the Class FDP from *Clostridium difficile* 630, was obtained by CryoEM-SPA collected at Titan Krios CM01–ESRF.

The different structures are under refinement and the results obtained and the structural comparison between the different classes will shed light on the molecular mechanisms associated with their response to the substrates ($O_2$, NO and $H_2O_2$) at a structural level.

**References**:

[1] Romão, C. V., Vicente, J., Borges, P., Frazão, C., Teixeira, M. The dual fucntion of flavodiiron proteins: oxygen and/or nitric oxide reductase, J Biol Inorg Chem, 2016, 21:39-52

[2] Folgosa, F., Martins M. C. and Teixeira M. The multidomain flavodiiron protein from *Clostridium difficile* 630 is an NADH:oxygen oxidoreductase, Scientific Reports , 2018, 8:10164

# Interlayer intercalation of 2-mercaptobenzothiazole in layered double hydroxides grown on AA2024 alloy: an *in situ* study at SOLEIL synchrotron

I. Sousa [1*], C. Neves [1], A. Salak [1], J. Tedim [1]

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Layered double hydroxides (LDHs) have been investigated as conversion films for protection of metal alloys and are suggested as prospective environmentally friendly candidates for chromate conversion coatings substitution on aluminium alloys. Previously, our group reported the successful growth of a thin multifunctional layer, with anticorrosion and biocidal properties, using LDH clusters as precursors.[1] Upon exchange with 2-mercaptobenzothiazole (MBT), more diffuse and asymmetric basal diffraction reflections of LDHs were observed, which was suggested to be a consequence of fragmentation of LDH crystallites during anion exchange. Yet, the mechanism of intercalation of MBT into the interlayer galleries of an LDH remained unclear. Literature suggests that multiple arrangements of MBT anions in the galleries may take place during the exchange process,[2] however we suggest that the $OH^-$ ions present in solution, may play an important role in facilitating intercalation of MBT into the galleries in the early stages of the exchange reaction.

Experiments were conducted at room temperature and MBT-containing solution was pumped into a reaction vessel containing a diluted LDH slurry or an AA2024 substrate with a grown LDH layer. Diffraction patterns were recorded during the first 80 min of the exchange reaction at every 2 s, starting immediately after injection, and afterwards at every 15 min for the next 120 min. The energy of the incident X-rays was 25 keV ($\lambda = 0.51359^\circ$). Data were collected in the 1.5–24° 2θ range. Obtained results suggest that the anion exchange occurs at a very early stage (first minutes) and that, over time, two different arrangements of MBT species corresponding to two basal spacing were observed. It was also confirmed that, no traces of the parent LDH–nitrate were detected after anion exchange.

**References:**

1. Neves, C.S. et al. (2019), *Coatings,* 9, 328.
2. Serdechnova, M. et al. (2016), *Journal of Solid State Chemistry*, 233,158-165.

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# Discovering morphology mapping with *operando* small-angle X-ray scattering 3D printing at ALBA

Daniel P. da Silva[1,*], Anabela P. Massano[1], João Matias[1], Marc Malfois[2], Paula Pascoal-Faria[1], Artur Mateus[1] and Geoffrey R Mitchell[1]

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**Abstract:** Polymer extrusion-based 3D printing is part of the set of technologies that enables the production of certain parts directly from their digital definition. In practice, the shape of the objects is obtained by successive layers of deposited molten polymer, and typically, this process focuses on geometry and dimension replication. However, it is also possible to look at 3D printing, also as a tool for material design along the fabrication of the final parts, since all processing parameters influence the final morphology of the materials.

Our team has been conducting studies about the influence of the main processing parameters in the final morphology of semi-crystalline thermoplastics. The idea is that it would be possible to attribute distinct properties along an entire part made of a single material, creating a concept introduced as morphology and/or property mapping.

To fully understand the process, we have been using a customed 3D printer which can be mounted directly on the ALBA NCD-Sweet beamline, allowing the elaboration of *operando* small-angle X-ray scattering (SAXS) trials, evaluating in real-time the structural development in the extrudates.

Despite some technical challenges in performing this sort of experiment with synchrotron radiation (to be discussed in this work), the latest works have shown some promising results and, ultimately, some potential benefits such as the reinforcement of recycled material, and the implementation of a new stage in design for additive manufacturing.

**Keywords:** 3D printing, polymer morphology, crystallization, morphology mapping, X-ray scattering.

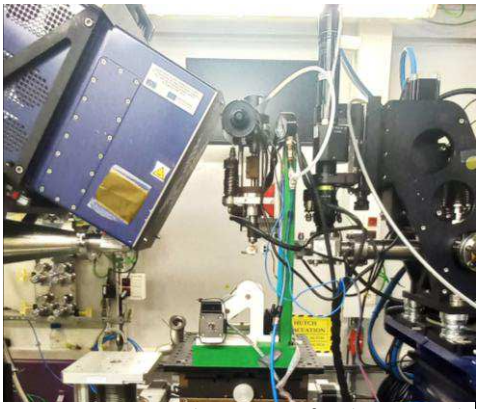

Figure 1 - Customed 3D Printer for the operando experiments on the NCD-SWEET beamline at the ALBA Synchrotron.

**Acknowledgements:** This work is supported by the Fundação para a Ciência e Tecnologia (FCT) through the Project references: MIT-EXPL/TDI/0044/2021, UID/Multi/04044/2013; PAMI-ROTEIRO/0328/2013 (Nº 022158), Add.Additive - add additive manufacturing to Portuguese industry POCI-01-0247-FEDER-024533 and UC4EP PTDC/CTM-POL/7133/2014). These experiments were performed at NCD-SWEET beamline at ALBA Synchrotron with the collaboration of ALBA staff.

# Pressure-induced transitions in 50 mol.% Fe-to-Sc substituted $BiFeO_3$ *via in situ* synchrotron X-ray diffraction study

**A. N. Salak[1*], J. P. Cardoso[1], D. D. Khalyavin[2], P. Fertey[3], A. Barbier[4]**

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We have recently found from the detailed study of the perovskite $BiFeO_3$-$BiScO_3$ system that an annealing the high-pressure synthesized phases results in the irreversible formation of polymorphs, which represent novel polar and antipolar structures with interesting magnetic properties [1,2]. We have shown [1] that the equimolar solid solution of this system ($BiFe_{0.5}Sc_{0.5}O_3$) exhibits an irreversible annealing-stimulated transformation: orthorhombic *Pnma* → rhombohedral *R*3*c* → orthorhombic *Ima*2. The polar *Ima*2 polymorph of $BiFe_{0.5}Sc_{0.5}O_3$ is a rare example of a polar weak ferromagnet with a non-collinear polar structure.

High-pressure synthesis is a powerful tool to obtain novel materials. Unfortunately, this technique is hardly upscalable and therefore cannot be used for industrial production. However, the compressively-strained epitaxial films of Bi-containing perovskites, which can be obtained in bulk form under high-pressure only, can be produced using the conventional film deposition techniques.

To estimate strains necessary for stabilization of epitaxial films of the polar *Ima*2 polymorph *via* thin-film strain engineering and to reveal other possible metastable multiferroic phases, we assessed the behavior of $BiFe_{0.5}Sc_{0.5}O_3$ under pressure *via in situ* synchrotron diffraction study over the range from ambient pressure to about 20 GPa at room temperature. The study was performed at the CRISTAL diffraction beamline of the national synchrotron SOLEIL (France) by means of micro powder diffraction using the membrane diamond anvil cell.

We have found that the orthorhombic *Ima*2 phase of $BiFe_{0.5}Sc_{0.5}O_3$ is stable up to about 2 GPa; then it transforms into the orthorhombic *Pnma* phase (antipolar). Both these polymorphs can exist at ambient conditions as metastable phases. Two new polymorphs, namely the orthorhombic *Imm*2 one (between about 2 and 8 GPa) and the orthorhombic *Pnma* (non-polar) (above 8 GPa) have been revealed.

[1] D.D. Khalyavin, A.N. Salak, *et al*., *Chem. Comm*. **55**, 4683 (2019).
[2] D.D. Khalyavin, A.N. Salak, *et al*., *Phys. Rev. B* **89,** 174414 (2014).

# Advantages of FTIR Microspectroscopy with Synchrotron Radiation - an Inside View into Osteosarcoma Treatment

Raquel C. Laginha [1], Jéssica D. Silva [1], M. Paula M. Marques [1,2], Gianfelice Cinque[3], Luís A. E. Batista de Carvalho [1], Ana L. M. Batista de Carvalho [1]

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Osteosarcoma (OS) is the most common primary malignant bone cancer with a poor prognosis for patients with metastatic or recurrent disease, with a greater incidence in children and young adults.[1] Hence, there is an urge to develop new and more effective anticancer agents while having minimal effects on healthy tissues. In the last 30 years, some progress has been achieved regarding OS therapy and survival rates have increased from less than 20% to 65-70% with the postoperative multidrug regimen designated as MAP (methotrexate (MTX), doxorubicin (DOX)) and cisplatin). However, since the severe toxicity associated with MAP is a limiting factor the currently ongoing European and American Osteosarcoma Study (EURAMOS-1) phase III clinical trial seeks to improve survival rate of OS patients through MAP concentration adjustments.[2] The advantage of a combined therapy is to be able to deliver the same or an enhanced cytotoxic effect relative to the one attained with each drug individually, with less deleterious side effects.

Vibrational microspectroscopy –FTIR with synchrotron radiation – was used to assess drug's bioavailability, biodistribution, metabolic impact and cellular response to treatment., Newly synthesized cisplatin-like compounds were tested ($Pd_2Spm$ and $Pd_3Spd_2$), both alone and in combination using the MAP regimen, against both osteosarcoma (cancer cells) and osteoblasts (healthy cells) cell lines.[3,4]

The results thus gathered clearly evidenced a spectral differentiation between the control cells, the cells treated with the MAP combination according to the EURAMOS-1 protocol – incubation with Pt(II)/Pd(II) drug at 4.8 $\mu$M + DOX at 3 $\mu$M for 72 h after which a dose of MTX at 4.8 $\mu$M was added for an additional 24 h period – and cells treated with Pt(II)/Pd(II) drugs at their $IC_{50}$ – cisplatin (12 $\mu$M), $Pd_2Spm$ and $Pd_3Spd_2$ (14 $\mu$M). Interpretation of the data was carried out through unsupervised PCA analysis of the spectra.

References:

1. S.U Lauvrak, E Munthe, S.H. Kresse, et al, Functional characterisation of osteosarcoma cell lines and identification of mRNAs and miRNAs associated with aggressive cancer phenotypes. Br J Cancer. 109 (2013) 2228-2236.

2. J.S. Whelan, S.S. Bielack, N. Marina, et al. EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. Ann Oncol. 26 (2015) 407-414.

3. T.J. Carneiro, A.S. Martins, M.P.M. Marques, et al, Metabolic Aspects of Palladium(II) Potential Anti-Cancer Drugs. Front. Oncol. 10 (2020) 1-8

4. A.S. Martins; A.L.M. Batista de Carvalho; I. Lamego; et al, Cytotoxicity of Platinum and Palladium Chelates against Osteosarcoma. ChemistrySelect. 5 (2020) 5993-6000.

# Deformation behavior and strengthening effects of an eutectic $AlCoCrFeNi_{2.1}$ high entropy alloy probed by in-situ synchrotron X-ray diffraction and post-mortem EBSD

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## Abstract

In this work, high energy synchrotron X-ray diffraction was used during tensile testing of an as-cast eutectic $AlCoCrFeNi_{2.1}$ high entropy alloy. Aside, from determining for the first time the volume fractions of existing phases, we further detail their role on the alloy deformation behavior. The two major phases, a soft disordered FCC and a hard ordered B2 BCC, were observed to exhibit a stress partitioning effect which can be used to modulate the mechanical response of the material based on the relative volume fraction of each phase. Dislocation density analysis revealed that the soft FCC phase had a significantly higher dislocation density right after the onset of plastic deformation. This is attributed to the existence of strain gradients across the lamellar structure, where the hard B2 BCC prevents free deformation of the FCC phase. Nonetheless, despite the increase of the dislocation density in the soft FCC phase, calculations of the strengthening effects induced by generation of dislocations are more significant in the hard B2 BCC phases, as this phase is primarily responsible for the strength increase in the alloy. Besides, the evolutions in dislocation density of the soft FCC and hard B2 BCC phases during tensile deformation obtained from synchrotron X-ray diffraction data are consistent with the evolution of KAM determined by EBSD characterization. Also, lattice strain analysis across two principal directions (parallel and perpendicular to the loading axis) reveals that for these specific orientations there is a preferential deformation of the hard FCC planes which can be related to the deformation response of specific lattice planes at distinct orientations, as well as to the phase partitioning stress behavior.

**Keywords:** Eutectic high entropy alloys; Synchrotron X-ray diffraction; Thermodynamic calculations; Mechanical testing; Digital image correlation.

# One step forward to understand the biological reduction of $CO_2$ to Formate by Mo/W Fdhs

**Guilherme Vilela Alves[1,2],** Ana Rita Oliveira[3], Cristiano Mota[1,2], Rita Rebelo Manuel[3], Kateryna Klymanska[1,2], Christophe Léger[4], Vincent Fourmond[4], Bruno Guigliarelli[4], Maria João Romão[1,2] and Inês Cardoso Pereira[3]

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The reversible interconversion of $CO_2$ into formate by Mo/W-Formate dehydrogenases (Fdhs) placed these enzymes on the spotlight, probing a promising route not only for green gas sequestration but also a sustainable way to produce fuel. Formic acid is a safe option for hydrogen storage/delivery (53g H2/L) in cell power applications [1].

FdhAB is a periplasmic heterodimer and the main responsible for $CO_2$ reduction in *D. vulgaris (Dv)* [2]. It comprises a Tuco cofactor in the active site (W-bisMGD, selenocysteine and a sulfido ligand) and four [4Fe4S] clusters responsible for electron transfer. Contrary to other Fdhs, this enzyme is oxygen-tolerant and can be purified aerobically [3]. Due to its robustness and high catalytic activity, *Dv*FdhAB is a suitable model for biocatalytic applications for $CO_2$ reduction.

Biochemical and structural studies on *Dv*FdhAB unveiled oxidized and reduced forms of the enzyme and unique features related to its robustness [3, 4]. The requirement for its pre-activation with reducing agents (DTT, TCEP or β-mercaptoethanol) led us to consider a putative role for a disulfide bridge 23 Å away from the active site. C845A and C872A mutants hinder the formation of this disulfide and were shown to be catalytically like the pre-activated wild-type enzyme in the absence of reducing agents, leading to the proposal that this

disulfide bridge might work as a Redox Switch for enzyme activation and O2 protection [5]. Structural studies disclosed relevant conformational changes promoted by the absence of the disulfide and results and mechanistic implications will be presented.

**Acknowledgments:** This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project PTDC/BII-BBF/2050/2020, and UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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# POSTERS

# Structural studies of bacterial PHA synthases

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**ABSTRACT:**

Polyhydroxyalkanoates (PHAs) are natural polyesters synthesized by bacteria that can be used in dif erent applicai ons, being a promising alternai ve for industrial use as a replacement of petroleum-based plasi cs. Due to remarkable characterisi cs, such as, biodegradability and biocompai bility, PHAs are also a suitable material for a wide variety of applicai ons as fabricai on of resorbable medical devices or drug encapsulai on.

Bacterial PhaCs enzymes catalyse the PHA polymerizai on step and are key in dei ning the biopolymer composii on and consequently its thermo-mechanical properi es. Nonetheless, PhaCs si ll require opi mizai on to produce PHAs with the desired mechano-chemical characterisi cs at a compei i ve price.

We heterologously expressed and characterized class I and II PhaCs from *Pseudomonas mandelii*. These enzymes are homodimers in solui on and present two domains (N-terminal and catalyi c domains). The N-termini domains seam to play a key role in dimerizai on and truncated forms of the enzyme do not present synthase aci vity. We aim at crystallizing and solving the crystal structure of PhaCs, that will enable ideni fying the molecular determinants for substrate specii city and mechanism in order to opi mize ei ciency and allow tailor made PHA produci on.

**Keywords:** Polyhydroxyalkanoates, Polyhydroxyalkanoate synthase (PhaC), X-ray crystallography, Bioplasi cs, Crystallizai on

# Structural insights into host glycoprotein recognition by *Bacteroides caccae*: the 3D Structure of a novel member of family 32 CBM in complex with the Tn Antigen

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**Keywords:** *human gut microbiota, Bacteroides caccae, carbohydrate-binding module, Tn antigen recognition, X-ray crystallography*

The human gastrointestinal tract harbors a diverse community of commensal bacteria, named gut microbiota. These microorganisms are beneficial to the human host, due to breaking down of dietary polysaccharides, such as starch, which cannot be digested. However, in a low-fiber diet, host glycans provided by the intestinal mucus, which is mainly composed of mucin *O*-glycosylated proteins, are used as an alternative source of energy. Therefore, in these conditions, the human gut microbiota has been associated with susceptibility to pathogens and the progression of intestinal diseases, owing to the destruction of the colonic mucous layer (Figure 1A)[1]. Thus, the study of these microorganisms and their binding to the host glycans may elucidate the role of the gut microbiota in these diseases. The commensal bacteria *Bacteroides caccae* has been reported to express different polysaccharide utilization loci (PUL), which encode for all the genes necessary for the breakdown and uptake of a given carbohydrate, such as those that code for M60-like metallopeptidases (Pept_MA) and their appended non-catalytic ancillary carbohydrate-binding modules of family 32 (CBM32)[2]. We have solved using X-ray crystallography methods the individual crystal structures of BC16100-C (a member of the CBM32 family appended to a Pept_MA from the *B. caccae* PUL-53) in its free form, bound to GalNAc and bound to the Tn antigen (GalNAcα-Ser) (Figure 1B). The 3D structures reveal insights on the amino-acid residues that recognize the carbohydrate ligands.

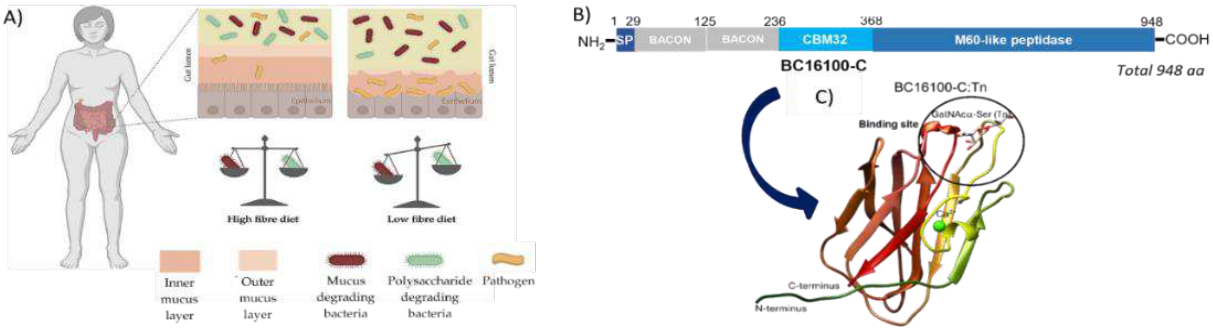


**Figure 1**: A) Impact of diet on the gut microbiota; B) Modular organization of PUL-53 from *B. caccae*; C) Ribbon representation of BC16100-C in complex with the Tn antigen (GalNAcα-Ser), colored from the N-terminus to the C-terminus. The Tn binding site is indicated by the black circle and the calcium ion is represented by the green sphere. The bound Tn is shown as sticks.

*Acknowledgements: Fundação para a Ciência e a Tecnologia for the project grants, RECI/BBB-BEP/0124/2012, PTDC/BIA-MIB/31730/2017, 2022.06104.PTDC, for funding the projects UIDB/04378/2020, LA/P/0140/2020, the fellowship SFRH/BD/143494/2019 (to FT) and the DL-57/2016 Program Contract (to BAP). AC holds a FEDER fellowship through program Portugal 2020 (project LISBOA-01-0247-FEDER-047033).*

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# Exploring Deinococcus arsenic resistance as a tool for bioremediation

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*Deinococcus* genus harbors extremely resistant species to high levels of γ/UV radiation as well as desiccation[1,2]. Besides this remarkable resilience to radiation, *Deinococcus indicus* is endowed with the ability to resist arsenic[3]. Arsenic is a widely distributed heavy metal with the most prevalent inorganic forms being trivalent arsenite [AS(III)] and pentavalent arsenate [AS(V)], both being highly toxic. Arsenic found in water system is easily absorbed by organisms causing high deleterious to health issues[4]. In this vein we aim to shed light on the mechanism for arsenate detoxification and the possible use of *Deinococcus* strains as a bioremediation tool of arsenic. Arsenate reductase (ArsC) is able to catalyze the reduction of As(V), and we are interested to study this protein from the *Deinococcus* genus. Here we report the expression, purification, and structural characterization of *D. indicus* ArsC. Moreover, we aim to understand at a cellular level, where and how these bacteria can store arsenate.

**Acknowledgements**

We thank the Fundação para a Ciência e a Tecnologia, Project- PTDC/BIA-BQM/31317/2017, Metals in the radiation resistance bacterium *Deinococcus radiodurans*, its role in protection up to future applications and the European Union's Horizon 2020 research and innovation programme under grant agreement No 857203- IMpaCT - Imaging life from Molecules to cells - building knowledge on Cryo-electron microscopy methodologies at ITQB NOVA – www.itqb.unl.pt/impact.

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# Impact of Metallodrugs on Cellular Membrane Lipids

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**Abstract for Poster Submission**

Cancer is well known as one of the most prominent causes of death worldwide, with new and improved chemotherapeutic strategies standing as an urgent clinical need. Despite the discovery of cisplatin (*cis*-$(NH_3)_2PtCl_2$) in the 1960's, the development of new cytostatic drugs with improved anticancer activity as well as reduced acquired resistance and minimal side effects still remains as a pivotal necessity, leading to numerous drugs being developed to this day [1,2]. Polynuclear chelates with polyamines and chloride leaving ligands are a specific class of DNA-damaging agents capable of selectively forming metal–DNA covalent interactions *via* an interplay not available to conventional mononuclear Pt-drugs (I cisplatin), which may translate into an enhanced therapeutic effect [3,4]. Additionally, these compounds may also interact with other cellular components besides DNA, such as the cytoskeleton, plasma and cell membranes. As it stands, the involved biomolecular interactions are a key factor to understand these drug's mechanism of action, which is of the utmost importance for the design of new and more effective anticancer agents.

Herein, the impact of a metal chelate on the structural behaviour of the cellular membrane was analysed using model lipids – phosphatidylcholine and phosphoethanolamine derivatives, glycolipids and cholesterol. The dinuclear paladium complex $Pd_2Spm$ (Spm=Spermine=$H_2N(CH_2)_3NH(CH2)_4NH(CH_2)_3NH_2$) [5] was assessed, as well as cisplatin (*cis*-$Pt((NH_3)_2Cl_2$) as a reference drug, by synchrotron-based IR spectroscopy in attenuated total reflectance mode (SR-FTIR-ATR), at the Diamond Light Source (UK).

The results thus obtained (both in the far and mid-infrared regions) clearly evidence an interplay between the dinuclear palladium Pd(II) complex and the model lipids. Additionally, striking differences were observed relative to the effect of the mononuclear Pt(II)-agent cisplatin. These data provide useful information on the drug´s impact on the cellular membrane, thus helping to develop new metallodrugs with improved intracellular bioavailability and therapeutic effect.

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# Improving the Oxygen tolerance in a highly active [NiFeSe] Hydrogenase

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**Keywords:** biohydrogen, $O_2$ tolerance, enzyme engineering

The [NiFeSe] hydrogenases are a subclass of the [NiFe] hydrogenases where a selenocysteine replaces cysteine as one of the Ni terminal ligands (Fig. 1a). They have high catalytic activities, namely for $H_2$ production, are more tolerant to $O_2$ than their [NiFe] counterparts and are less inhibited by $H_2$. Nevertheless, they are also susceptible to inactivation by $O_2$: in the [NiFeSe] enzyme from *D. vulgaris* Hildenborough, our previous work showed this to arise from a reversible chemical oxidation of the proximal iron-sulfur cluster together with an irreversible oxidation of the terminal Ni ligand Cys 75 to sulfinate [1].

In addition to the well-known hydrophobic tunnel system that connects the active site with the molecular surface, a second tunnel system has been identified that is probably responsible for $O_2$ access to Cys 75. About halfway from the active site to the molecular surface, this tunnel is divided into a hydrophilic and a hydrophobic branch that continue to the surface (Fig. 1b).

By mutating residues in the large subunit that are located on that tunnel system, variants were produced, aiming to block $O_2$ access to Cys 75 and thus improve the $O_2$ tolerance of this enzyme. Two of these variants, where a Gly 491 in the large subunit was mutated to Ala and Ser residues were shown to successfully protect cysteine 75 against $O_2$ attack. Electrochemical and biochemical activity assays of these variants revealed an increase in $O_2$ tolerance in comparison with the wild-type enzyme [2].

However, this increase in $O_2$ tolerance was achieved at the cost of a significant reduction in activity (by as much as 50% in both $H_2$ evolution and uptake), probably due to steric hindrance of the added sidechain towards the mobility of conserved residue Glu 28, believed to be the first proton acceptor off the active site during catalytic turnover of the enzyme (Fig. 1c) [2]. Also, a study where crystals were pressurized with Kr and $O_2$ at more than 50 bar did not highlight any additional hydrophobic channels leading to the active site or Cys 75 [3].

Therefore, additional [NiFeSe] hydrogenase variants are being designed and characterized both structurally and biochemically, aiming to protect Cys 75 from $O_2$ attack with the consequent enzyme inactivation, while avoiding such a large impact in its activity. The latest results from these studies will be presented.

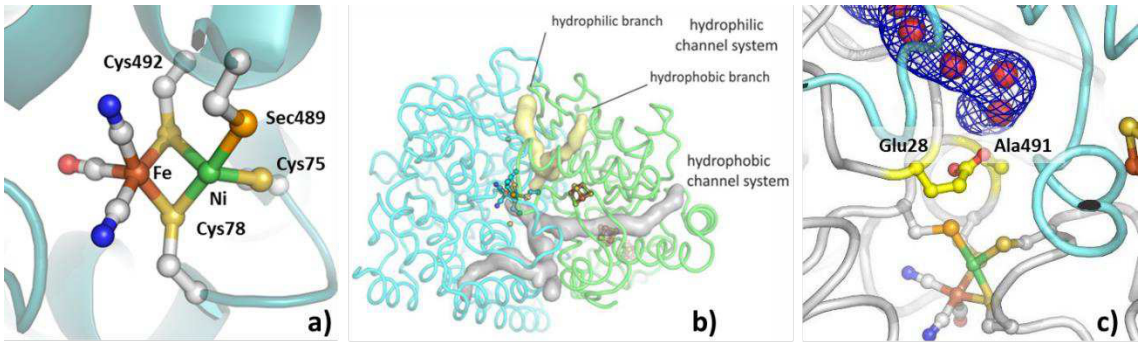


**Figure 1.** a) The active site in the anaerobically purified and crystallized [NiFeSe] hydrogenase from *D. vulgaris* Hildenborough; b) The channel systems in the [NiFeSe] hydrogenase; c) The solvent-filled hydrophilic channel in the G491A variant does not reach Cys75.

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POSTER ABSTRACT

# *Water dynamics in healthy-to-cancer transition: Insights from Quasi-Elastic Neutron Scattering*

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## Abstract

Cancer is a worldwide health problem, being the second leading cause of death globally – 9.6 million deaths in 2018 and expected to rise up to 22 million cases per year within the next two decades. The transition from normal to cancer (NTC) is still a poorly understood process, known to be closely associated to cellular biomechanical properties. Apart from the known biochemical/metabolic changes associated to malignancy, carcinogenesis is recognised to be intimately related to the cell´s biomechanical profile, in particular to intracellular water dynamics [1-3]. In this work, quasi-elastic neutron scattering (QENS) was used to probe the dynamics of water in (1) human cells (breast, prostate and lung), both malignant and non-malignant; and (2) human tissues (breast and tongue), cancer and respective surrounding normal specimens. An increased plasticity of the cytomatrix was observed upon normal-to-malignant transformation, the lung carcinoma cells displaying the highest flexibility followed by prostate and breast cancers. Additionally, different dynamics were found for malignant and non-malignant tissue samples, depending on their characteristics: a higher plasticity for breast invasive cancer tissue *versus* normal, and an opposite profile for tongue. This biophysical description of malignancy will hopefully shed new light onto the poorly understood NTC transition, which should contribute for the development of improved diagnosis and chemotherapeutic tools thus benefiting cancer treatment and oncology patients' prognosis.

***Keywords*** – normal-to-cancer transition (NTC); quasi-elastic neutron scattering (QENS); human cancer cells; human cancer tissues

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# Novel Biobased Composites of Polybutylene succinate with nano-sized carbonaceous fillers

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Keywords: Circular economy; polybutylene succinate; graphene nanoparticles; small-angle X-ray scattering.

Abstract:

The European strategy for plastics in a circular economy underlines the importance of using renewable polymers for sustainable applications. In this goal, the development of biodegradable and compostable materials as an alternative to fossil-based plastics is of paramount importance.

Recently, our team has been conducting studies with the aim of exploring the properties and processability of biobased composites of polybutylene succinate (PBS) with nano-sized carbonaceous fillers. Experiments performed at ALBA NCD-SWEET Beamline using small-angle X-ray scattering to analyze prepared extruded strands of polybutylene succinate/graphene composites revealed different morphology and degree of crystallinity in these materials.

Polybutylene succinate is a class of biodegradable aliphatic polyesters that can be synthesized from succinic acid and 1,4 butanediol. Nevertheless, due to its limited Young's modulus as well as its susceptibility to sudden degradation during melt processing, especially at high temperature, PBS is often blended, and reinforced with other polymers, fillers and additives to tackle the issues of better processability, higher stiffness, and improved overall mechanical strength. Thus, recent advances in polymer blending processes have rendered PBS blends an interesting material platform for applications that require a balance of mechanical strength and flexibility as well as thermal resistance together in compliance with industrial compostability standards. This presentation is part of a broad work focused on enhancing those properties through preparing composites with graphene nanoflakes 12 nm thickness blended with other biopolymers.

The addition of graphene nanoflakes will serve to enhance de mechanical and toughness of the material but also the graphene nanoflakes will serve to nucleate crystals and hence define the morphology.

The overall objective of this work is to draw correlations between the addition of graphene nanoparticles (GPN) in the extrusion-based processing parameters and the lamellar crystal orientation of extruded samples of PBS and PBS/GPN biobased composites.

Acknowledgements: This work was financially supported by the Fundação para a Ciência e a Tecnologia FCT/MCTES (PIDDAC) through the following Projects: MIT-EXPL/TDI/0044/2021, UIDB/04044/2020; UIDP/04044/2020; Associate Laboratory ARISELA/P/0112/ 2020; PAMI - ROTEIRO/0328/2013 (Nº 022158), plus EcoPlast, Materiais compósitos eco-sustentáveis para substituição dos plásticos convencionais, ref POCI-01-0247-FEDER-069002, and INNOV-AM funded by National Agency of Innovation. The small-angle X-ray Scattering measurements were performed at the ALBA Synchrotron Light Source in partnership with ALBA Staff.

# Nylon 7,10: Real time X-ray diffraction and FTIR microspectroscopy studies on a novel odd-even polyamide

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Linear aliphatic polyamides, also commercially known as nylons, are a family of synthetic polymers bearing amide groups in the macromolecular chain. The possibility to form hydrogen bonds gives rise to the well-established properties of these materials, from chemical, thermal and mechanical points of view. Nylons can be synthetized by the condensation of a monomer bearing both the acidic and amino terminals, or by the reaction of two monomers, containing the two complementary reactive functions. By the introduction of an odd-numbered carbon length monomer, it is possible to obtain odd-even or even-odd polyamides (e.g. nylon 4-9, 6-5, 5-6, 5-10, 4-7 etc.). These polymers revealed peculiar structural characteristics with respect to the commercially available even-even nylons. Following these reasons, a new type of odd-even polyamide, called nylon 7,10, was synthetized, by the interfacial polycondensation of 1,7-diaminoheptane with sebacoyl dichloride. The presence of sebacoyl units confers a partially bio-based character since they can also be produced from vegetal sources. Nylon 7,10 has been characterized by chemical (GPC, FTIR, and $^{1}$H-NMR) and thermal analysis (DSC and TGA). Wide angle X-ray diffraction (WAXD) and FTIR Microspectroscopy (SR-FTIRM) were performed at the ALBA synchrotron (*Cerdanyola del Valles, Barcelona*) to analyze both crystalline structure and the spherulitic morphology. Real-time WAXD measurements allowed evaluating thermal induced transitions and the non-isothermal crystallization. The evolution during a typical heating run is displayed in Figure 1a. Two features are significant: a) The initial crystalline structure is characterized by reflections around 0.440 nm and 0.380 nm, which may be associated to structures with a single hydrogen bonding direction (as presumable for even-even nylons) or a structure with two hydrogen bonding directions (as postulated for different odd-even and even-odd nylons), b) A Brill transition towards a pseudohexagonal form. Cooling runs demonstrated the apparition of an additional crystalline structure that is stable up to room temperature and is defined by a reflection around 0.455 nm (Figure 1b). By SR-FTIRM infrared spectra could be collected over 6x6 $\mu m^2$ regions, on spherulites previously crystallized from polymer films sandwiched between $CaF_2$ cover slides. These measurements were useful to analyze the microstructure of the spherulites, and the creation of chemical images of the sample (Figure 2) which reflected an orientation of characteristic bonds (e.g., amide A, amide I and amide II).

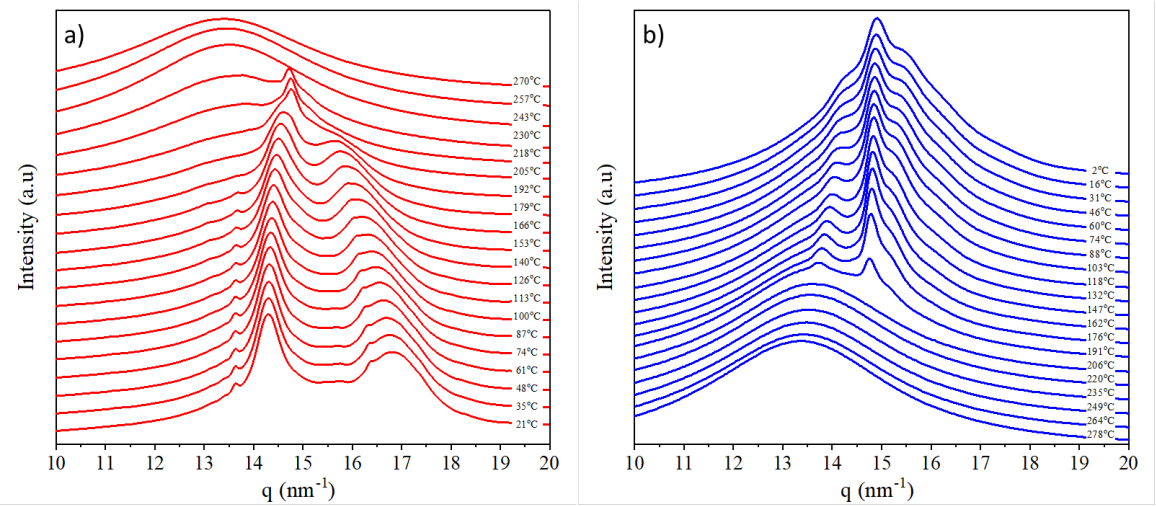


***Figure 1.*** *XRD profiles of Nylon 7,10 during heating (a) and cooling (b)*

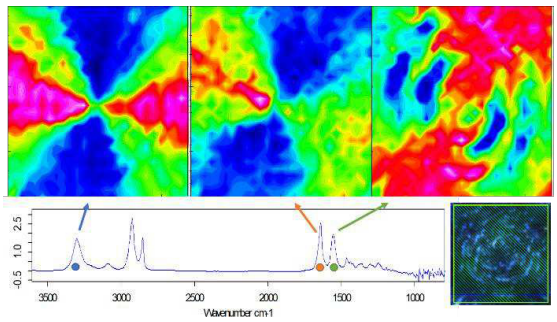


***Figure 2.*** *Top: SR-FTIRM chemical images of a ringed spherulite, obtained by integrating amide A (blue dot), amide I (orange dot), and amide II (green dot) signals. A clear orientation is visible in all images. Bottom: FTIR spectrum and the mapped spherulite.*

# Discovering Morphology Mapping with 3D printing at the NCD-SWEET Beamline at ALBA Synchrotron

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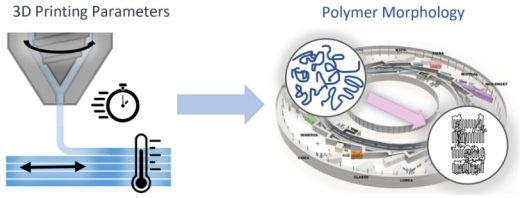
## GRAPHICAL ABSTRACT




## Introduction

Direct digital manufacture is a production technique that enables parts to be fabricated directly from their digital definition, allowing mass customization in several areas, including biomedical applications [1, 2].

In 3D Printing, the majority of developments have been focused on object shape reproduction. Some attention has been given to the process parameters in order to optimize polymer melt flow rate [3]. The influence of the manufacturing input variables on the mechanical properties of the prints, as with all polymer processing technologies, is also known [4]. An example of that is the shear and elongation of the polymer melt flow passing through a restricting die [5]. However, there is not a significant effort to design materials themselves during additive manufacturing.

This work is focused on this issue, envisioning the complete digital definition of produced part, from geometry to material properties. In this work, in situ time-resolving small-angle X-ray scattering measurements were performed at the ALBA Synchrotron Light Source, in Barcelona. Resorting to a customized 3D printer, the aim was to evaluate the possibility of morphology control of semi-crystalline polymers during extruder-based 3D printing. The ultimate goal of the project is to create a methodology to realise patterns of material properties, specifically the modulus of the polymer, along the printed parts.

## Experiment Design

The replicate the extrusion-based 3d printing operation at the NCD-SWEET Beamline, the designed equipment comprises three main parts: feeder, extruder, and a rotating collector. The model presented in Figure 1 simulates fabrication along a moving platform, with a theoretically infinite path (following the perimeter of the main collector roll). It makes use of a single extruder with a dual channel screw forcing the melt flow through a 300 µm inner diameter needle with a high length to diameter ratio (L/D ≈500).

The apparatus allows the control of the extrusion temperature, extrusion velocity, and write velocity (print speed). The extruder is fed with a melt from a pellet reservoir, and a compressed air source is required to feed the system.

Input instruments, such as the power supply, the heater-controlling modules, and the computer for remote control were placed separately of the beamline platform.

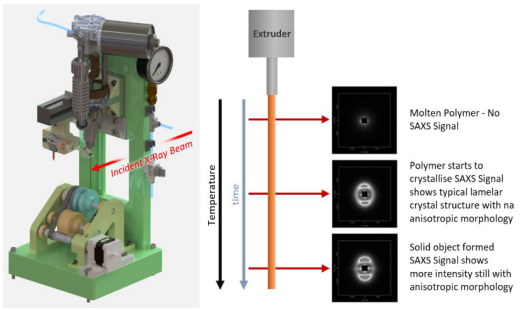



**Figure 1** – Left: a CAD representation of the 3D printer developed for this work. Right: a schematic of the quasi-static state of the extrudate in a constant gradient of temperature. The evolution of the structure can be evaluated by moving the incident X-ray beam down the jet. Adapted from [6].

## Materials and Methods

Samples Material:

In this experiment, the team was able to print with polycaprolactone (PCL) and low-density polyethylene (LDPE). The work in this poster is focused on the PCL samples. The material was supplied in the form of small pellets (~3mm) with an $M_w$ = 50,000 by Perstorp (Cheshire, UK).

Experimental Procedure:

Analysing the samples as they were being printed required a constant temperature profile of the extrudate filaments. Thus, the extrudates were observed in a quasi-steady state, in which the development of the structure and the morphology with time could be assessed by moving the platform up and down; repositioning the incident x-ray beam along the extrusion axis (as represented in Figure 1). Remotely controlled, each trial consisted of a scan of 20 steps of 0.1mm with a 1-second data collection period.

In Figure 2, it is possible to observe the equipment mounted on the beamline platform.

## Results

The most expressive result can be observed in Figure 3, which shows 2 rows of SAXS patterns. The bottom patterns are related to extrudates produced at a higher write speed.

As it is possible to notice in the right-hand pattern, the solidified filaments produced at a low speed have a broadly isotropic crystal orientation; whereas the equivalent pattern in the bottom, row reveals a high level of orientation (as indicated by the two sharp maxima above and below the zero-angle point).

The pronounced level of orientation of the lamellar crystals shown in the bottom row of SAXS patterns is very typical of row-nucleated crystallisation, when the row nuclei generate a common and highly aligned templating mechanism. This behaviour is likely due to the fact that the higher write speed leads to faster cooling, once the extruded material is moved away from the hot end of the extruder more rapidly. This means that crystallisation should occur before the extended chains have totally or completely relaxed. This phenomenon, according to other studies [7], can be related to the increase of the elastic modulus by more than a factor of 2.

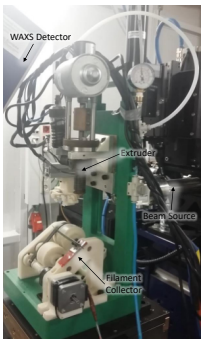



**Figure 2** – Customized 3D Printer at the NCD-SWEET Beamline of ALBA Synchrotron.

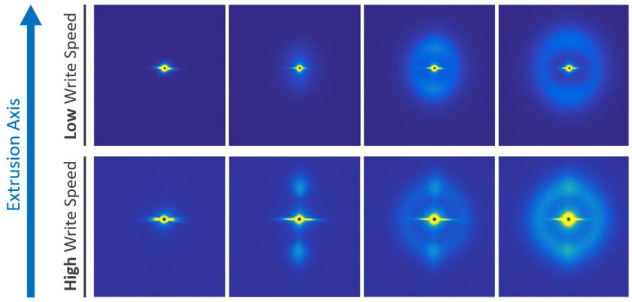



**Figure 3** – SAXS patterns of the PCL extrudate measured with the incident X-ray beam positioned at increasing distances from the exit of the extruder die (x = 0 mm). The left-hand scans are the closest to the extruder die, and those to the right are positioned further away. For all of these images, the 3D printer parameters were fixed for extrusion temperature, extrusion speed, and ambient temperature. The scans from the top line correspond to a low write speed, and the bottom scans to a high write speed. Adapted from [6].

## Conclusions and Future Work

The 3d printer worked successfully, and it was shown that is possible to control the processing parameters to produce deposited polymeric material with different morphologies that exhibit different physical properties.

Varying the input parameters in real-time during manufacturing provides a method of depositing the polymer with distinct properties in different zones of a printed part.

With the 3D printing in situ studies of time-resolved X-ray scattering, it was possible to establish some correlations between the printing conditions and the level of preferred orientation in the deposited material.

Future developments are expected, especially regarding the design exploration of products to take advantage of this new approach.

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# Experimental setup and equipment modelling for in-situ morphology SAXS/WAXS measurements of electrospun polymer fibres on the NCD-SWEET Beamline at the ALBA Synchrotron Light Source

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**Abstract:** Extensive research has been conducted on the utilization of nanofiber production in various applications, including drug delivery, tissue engineering, and composite materials for high structural performance. Electrospinning is a prominent technique, offering a relatively straightforward approach to achieving uniformly layered polymer fibers.

A polymer solution is dispensed from the tip of a needle with a high-voltage charge. This results in the expulsion of a stream of strands onto a rotating platform, creating a thin coating on a substrate in the collector's surface.

The electric field pushes the strands toward the collector, and as they solidify, the ultimate material morphology, as well as the stability of the flow direction of the nanofibers significantly depend on the process parameters. Understanding how these parameters influence the material flow and crystallization is fundamental to establishing a correlation between the process conditions and the final properties of the polymers. An effective approach to study this correlation is by analyzing the fibers using X-ray scattering while they are being electrospun.

This work presents an experimental methodology, highlighting the main challenges, risks, and procedures involved in carrying out electrospinning in the ALBA synchrotron NCD-SWEET beamline.

Our team developed an electrospinning apparatus with a rotating drum collector, allowing for real-time scanning of the samples, and addressing additional variables to ensure the feasibility of the experiment while maintaining the safety and normal operation of the beamline facilities. This discussion mainly focuses on the limitations and feasibility of these measurements.

**Keywords:** electrospinning, polymer morphology, crystallization, X-ray scattering.

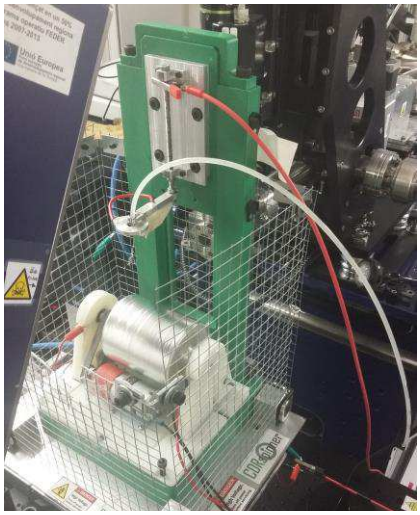

Figure 1 – Electrospinning equipment on the beamline platform.

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

# ENURS 21ST JUNE 2023

ENURS 2023 - 9th Annual Meeting of Portuguese Synchrotron Users showcased disruptive developments and collaborative opportunities in their field of synchrotron research.
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The event featured insights into the European Synchrotron Research Facility in Grenoble, France, renowned for state-of-the-art infrastructure and pioneering research.

Additionally, the Alba Synchrotron Light Source in Barcelona, Spain, revealed fascinating advancements, enhancing our understanding of materials and molecular structures. Participants engaged in vibrant oral and poster presentations, fostering discussions and constructive feedback.Networking sessions facilitated new connections and collaborations.

Esteemed keynote speakers inspired attendees with their profound insights and vision for the future.The event celebrated excellence with an award ceremony, honoring individuals and teams who have made significant contributions to advancing knowledge.ENURS 2023 had a lasting impact on the field of synchrotron research, fostering connections and shaping the future of scientiffic progress.


# AWARDS ENURS 2023

## Poster awards

**1° Filipa Engrola (UCIBIO/NOVA)**
"A biophysical and structural approach to shed light on arsenite oxidase reaction mechanism."

**2° Anabela Massano (CDRSP/IPLeiria)**
"Novel Biobased Composites based on Polybutylene succinate with nano-sized carbonaceous fillers."

**3° Daniel Silva (CDRSP/IPLeiria)**
"Discovering Morphology Mapping with 3D printing at the NCD-SWEET Beamline at ALBA."

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