2nd National Crystallographic Meeting

Lisbon, Portugal

15 – 16 July 2022

Instituto Superior Técnico

Departamento de Engenharia Química

Lisboa, 15 - 16 July 2022

BOOK of ABSTRACTS

WELCOME MESSAGE

Dear Colleagues,

The Second National Crystallographic Meeting will be held in Lisbon, at Chemical Engineering Department of Instituto Superior Técnico, Lisbon University, in the 15th and 16th of July, three years after our first Meeting in 2019 in Aveiro

We are now out of restrictions of COVID-19 pandemic, so this Meeting will be held in a presential mode.

This Second National Crystallographic Meeting aims to bring together all scientists, from different areas of knowledge, working in Diffraction and Crystallography in Portugal. We want to maintain a forum for discussion and exchange of innovative ideas and prospects for the future of Crystallography in Portugal.

A preliminary programme schedule is proposed, and it will soon be completed with the names of the Scientists delivering the Plenary Lectures. As for the oral presentations we need your Abstract to be submitted, as they will be chosen from there.

The attendance of students is strongly encouraged, as we they are the future of the Scientific Area in the country.

We look forward to welcoming you in Lisbon.

On behalf of the Organizing Committee

Teresa Duarte

COMMITTEES

Scientific Committee

- Chair: Teresa Duarte (IST-ULisboa)
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- Clara Gomes (FCT- NOVA)
- Isabel Santos (IST- ULisboa)

GENERAL INFORMATION

Registration

The registration fee includes:

- Admission to all the Meeting’s scientific sessions
- Conference materials
- Coffee breaks
- Conference lunch

Poster and oral presentations (technical information)

Plenary sessions will have 30 minutes plus 5 additional ones for discussion.

Oral session will have 12 minutes plus 3 additional ones for discussion

All speakers should check with the Organization at least one hour prior to the beginning of their session to test and deliver the presentation.

The dimensions of the poster presentations should not exceed A0 (84 cm x 118 cm).

Please note that the Organization will not be responsible for the posters that are left in the panels after the session.

Official language

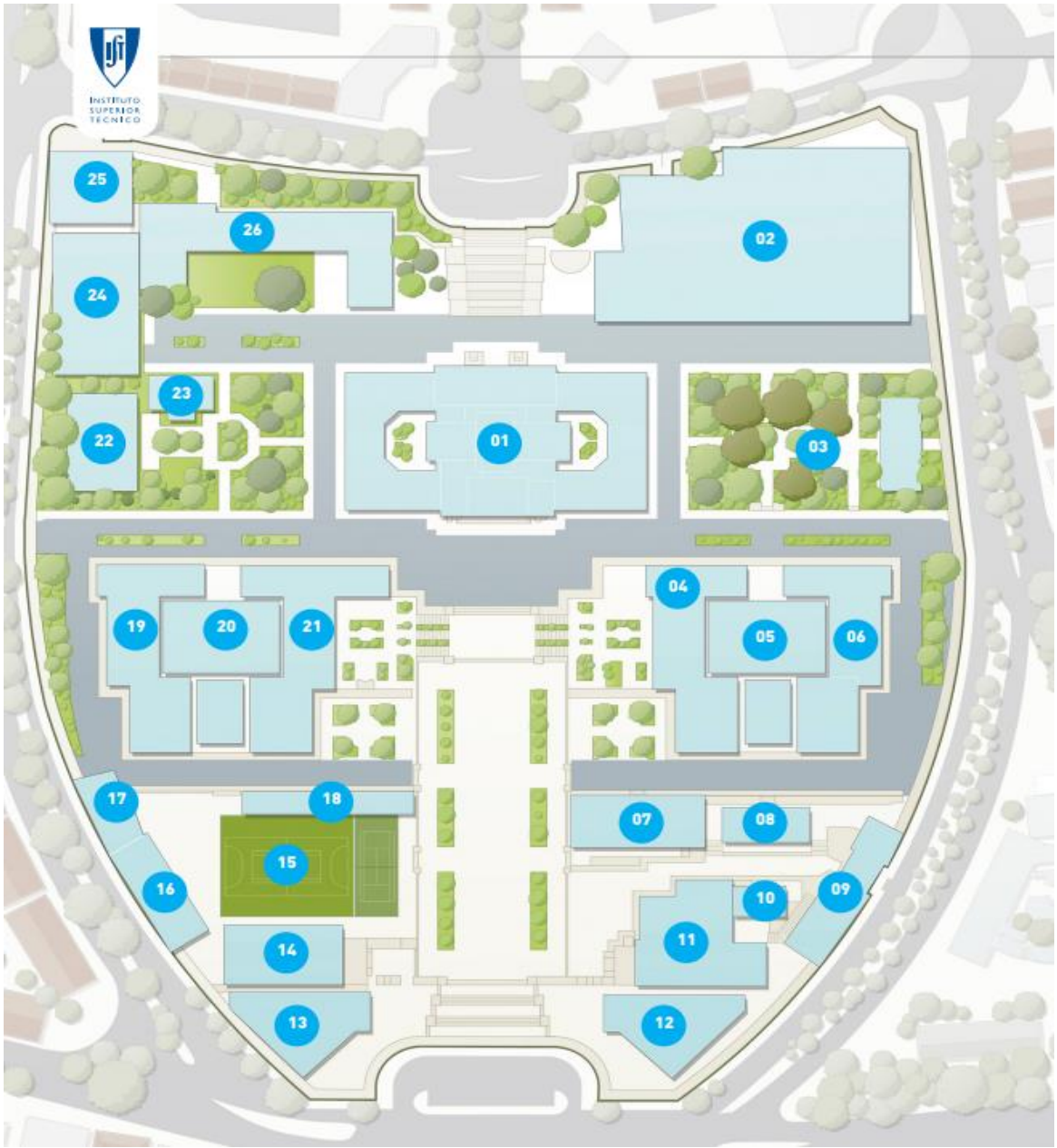
The official language of the Workshop is English. No simultaneous translation will be provided.

Badges and Security

It is essential that you always wear your personal badge while in the Workshop venue and during all the Events, as it is the official entrance pass to scientific sessions and other activities.

VENUE MAP

The location of all conference and poster session will be at the South Tower, **building number 20**



Schedule

	Friday 15.07
13h30	Registration
15h00	Opening
15h10	**PL1- Richard Garratt -** *Molecular recognition within septin filaments - integrating protein crystallography and cryo-EM*
15h45	**PL2 -Franziska Emmerling -** *Shaken not stirred: enhancing the flavor of mechanochemistry*
16h20	Coffee Break
16h45	**OC1 - José Brito -** *Structural and functional insights into hydrogen sulfide homeostasis in pathogenic bacteria*
17h00	**OC2 - Pedro Pereira -** *Flexible, decorated and tight - a new breed of natural anticoagulants*
17h15	**OC3 - Elin Moe -** *Base Excision Repair (BER) in the extremely radiation resistant bacterium D. radiodurans – a crystallographic view*
17h30	**PL3 - Daren Fearon -** *From crystallographic fragment screen to preclinical candidate: Open science discovery of SARS-CoV-2 antivirals*

	Saturday 16.07
09h00	**PL4 - Delia Haynes -** *Dithiadiazolyl radicals as building blocks for functional materials*
09h35	**OC4 - Clara Gomes -***Ar-BIAN-Zinc-based complexes: synthesis, structural characterization, and application as catalysts for cyclic carbonates formation*
09h50	**OC5 - Adelino Galvão -** *Supramolecular structures of camphor sulfonimines*
10h05	**OC6 - Ricardo Mendes -** *Enhancing Metal-Organic Frameworks by Post-Synthetic Modification*
10h20	Coffee Break
10h45	**OC7 - Filipa Engrola -** *Arsenite oxidase – a complex enzyme to tackle a complex environmental problem: arsenite water contamination*
11h00	**OC8 - Rute Chaves -** *Archaeometric Study of Chalcolithic Ceramics from Lisbon*
11h15	**OC9 - Pavel Zelenovskii -** *Energy landscape and piezoelectric properties of diphenylalanine nanostructures*
11h30	Poster Session and Social Lunch
14h30	**OC10 - Mathilda L. Coutinho -** *A XANES approach to the blue and yellow pigments in ceramic heritage*
14h45	**OC11 - Inês Martins -** *Jumping from crystalline to amorphous drugs: How can we understand their molecular-level organization?*
15h00	**OC12 - Guilherme Alves -** *Formate dehydrogenase presents a retention cavity in the substrate tunnel*
15h15	**PL5 - Cesar Santiago -** *Microcrystal Electrondiffraction (MicroED) implementation at CNB: making a short story long*
15h50	Closing and poster/oral prizes
16h00	Farewell Coffee

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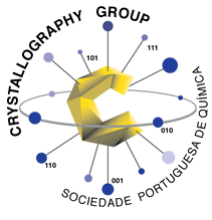
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PL4 – Dithiadiazolyl radicals as building blocks for functional materials

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Oral Presentations

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OC6 – Enhancing Metal-Organic Frameworks by Post-Synthetic Modification

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OC12 – Formate dehydrogenase presents a retention cavity in the substrate tunnel

POSTER

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P2 - Structural analysis of tree distinct classes of Flavodiiron proteins obtain by X-ray Crystallography and Single-Particle Cryo-EM

P3 - Structural details for mucin-type O-glycan recognition by Bacteroides thetaiotaomicron

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P20 - Nitrate in Two Copper(I) Complexes: Ligand or Counterion?

P21 - From Cu2+ to Li+ Fluorescent Chemosensors

PLENARY PRESENTATIONS

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PL1

Molecular recognition within septin filaments – integrating protein crystallography and cryo-EM

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Septins are filament forming GTPases which are often considered to be the fourth filamentous component of the cytoskeleton. They play both passive roles (as membrane barriers) as well as active ones in a series of cellular events which often involve membrane remodeling (cytokinesis for example). For this reason septin filaments and their higher-order structures are capable of recognizing membrane curvature.

In humans, septin filaments form spontaneously by the polymerization of oligomeric particles which are composed of either three or four different subunits. A palindromic arrangement of these subunits leads to the appearance of either hexamers (if three subunits are involved) or octamers (if four). This requires subtle molecular recognition which gives rise to the formation of the correct interfaces, necessary for the assembly of the oligomers. These interfaces are of two types, called G and NC, which alternate along the filament.

Over the course of the last few years we have accumulated a large amount of crystallographic data allowing us to better understand this phenomenon. For the most part this has involved a “divide and conquer” approach to studying individual interactions. The full oligomers are more challenging as their intrinsic flexibility makes it difficult to obtain well-diffracting crystals. In order to overcome this we have resorted to cryo-EM which has provided significant new insights.

The accumulated data has allowed us to shed light on how the filament bends, how it interacts with membranes, on the special roles played by the different subunits and how selective interfaces are formed. Also, we provide a mechanism by which the hydrolysis of GTP at a G-interface induces structure rearrangements at the neighbouring NC-interface and its biological relevance. Finally, studies of the C-terminal domain alone (which is involved in the formation of coiled coils) allows us to build a picture of how higher order structures are formed and how their disruption by the Zika virus protease may be involved in events leading to microcephaly.

Acknowledgements

We thank the Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP) for financial support.

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PL2

Shaken not stirred: enhancing the flavor of mechanochemistry

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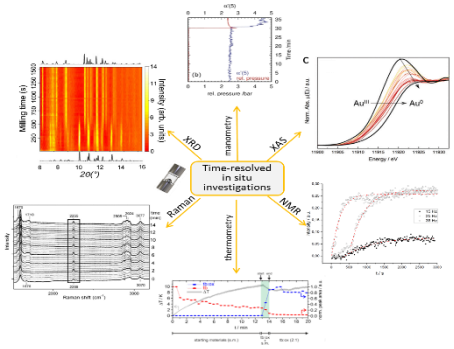
Mechanochemistry is increasingly used for synthesizing soft matter materials including metal-organic compounds and cocrystals.[1] The ever-increasing interest in this method is contrasted by a limited mechanistic understanding of the mechanochemical reactivity and selectivity. Different milling parameters are known to affect the mechanisms and rates of product formation: milling frequency, milling time, filling degree of the milling jar, ball diameter and vessel size, degree of milling ball filling, and material of jars. Time-resolved in situ investigations of milling reactions (Figure 1) provide direct insights into the underlying mechanisms.[2,3] We recently introduced different setups enabling in situ investigation of mechanochemical reactions using synchrotron XRD and XAS combined with Raman spectroscopy and thermography.[2,4] The presented setup allows the detection of crystalline, amorphous, eutectic, and liquid intermediates. Furthermore, the chemical composition of the reaction mixture was found to be directly correlated with changes in the temperature profile of the reaction. The resulting deeper kinetic and thermodynamic understanding of milling processes is the key to future optimization of mechanochemical syntheses. In this contribution, we will discuss our recent results investigating the formation of (polymorphic) cocrystals and coordination polymers.[5–8] Our results indicate that time-resolved in situ investigations of mechanochemical processes are key for tuning and optimizing mechanochemical syntheses allowing to unleash the potential of mechanochemistry for a green materials design.



Scheme or Figure 1: Established methods for time-resolved in situ investigations of mechanochemical reactions.

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[3] A. A. L. Michalchuk, F. Emmerling, *Angew. Chem. Int. Ed.* **2022**, *n/a*, DOI 10.1002/anie.202117270.
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From crystallographic fragment screen to preclinical candidate: Open science discovery of SARSCoV2 antivirals

D. Fearon [I,II]

[I] *Diamond Light Source, Didcot, United Kingdom,* [II] *Research Complex at Harwell, Didcot, United Kingdom*

The development of novel, low cost and globally available antiviral therapeutics remains an essential goal for the current SARSCoV2 pandemic. Furthemore, future pandemics could be prevented with easily deployable broadspectrum oral antivirals and open knowledge bases that derisk and accelerate novel antiviral discovery and development.

To identify starting points for the development of such therapeutics, the XChem team at Diamond Light Source, in collaboration with various international colleagues, performed large crystallographic fragment screens against 8 key SARSCoV2 protein targets including the Main protease[1], the Nsp3 macrodomain[2] and the helicase Nsp 13[3]. The expeditious collection and open dissemination of the data from these fragment screening campaigns was enabled by the well-established platform at Diamond Light Source and by the implementation of various experimental and computational tools. This work identified numerous starting points for the development of potent antiviral therapeutics as exemplified by the COVID Moonshot a fully open-science structure-enabled drug discovery campaign targeting the SARSCoV2 main protease.[4] By leveraging crowdsourced medicinal chemistry design, high throughput structural biology, machine learning and exascale molecular simulations we discovered a novel chemical scaffold that is differentiated to current clinical candidates in terms of toxicity and pharmacokinetics liabilities, and developed it into orally bioavailable inhibitors with clinical potential within 2 years.

All compound designs, structural data, assay data and synthesized molecules have been shared rapidly and openly, creating a rich, IP free knowledgebase for future anticoronavirus drug discovery.

1. Douangamath, A. *et al*., Nat Commun., 11, 5047 (2020).
2. Schuller, M. *et al.*, Science Advances, 7 (2021).
3. Newman, J. A., *et al.*, Nat Commun., 12, 4848 (2021)
4. The COVID Moonshot Consortium, *bioRxiv*, 10.1101/2020.10.29.339317 (2020)

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PL4

Dithiadiazolyl radicals as building blocks for functional materials

Delia A. Haynes[1,*]

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The 1,2,3,5-dithiadiazolyl radicals (DTDAs), R-CNSSN•, are of considerable interest due to their potential as building blocks for materials with interesting magnetic or conducting properties.[1] DTDAs are thermally and kinetically stable, but frequently dimerise in the solid state *via* an interaction known as pancake bonding,[2] rendering them diamagnetic. We are investigating a number of ways of overcoming this dimerization in order to develop materials with unique physical properties.

We have explored co-crystal formation as a way to overcome dimerisation in DTDAs.[3] Several DTDA co-crystals have been characterised by our group and others. Experimental charge density studies[4] as well as computational investigations have shed some light on the nature of the dimerisation interaction in these co-crystals. In related studies, we have shown that co-sublimation of DTDAs allows for control of the morphology and polymorphic form of a monomeric DTDA radical.

We have investigated the inclusion of DTDAs in porous materials. PhDTDA has been included in a metallocyclic host, where it is present as a monomer, and is stable in the host under ambient conditions.[5] We have also studied the coordination of DTDAs to metalloporphyrins.[6] It is clear that DTDAs show great potential as building blocks in the construction of molecular materials.

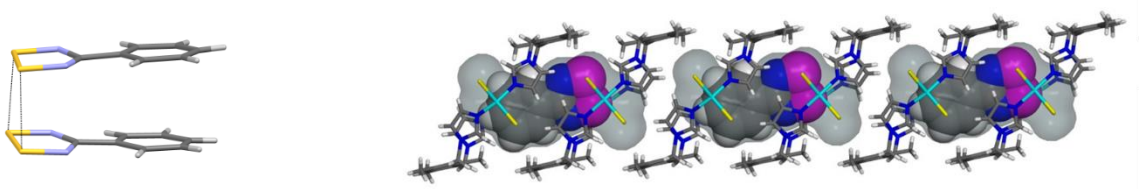

Figure 1: (left) A DTDA pancake-bonded dimer. (right) DTDA included in a porous metallocycle.

Acknowledgements

We thank the NRF and Sasol for funding.

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5. V. I. Nikolayenko, L. J. Barbour, A. Arauzo, J. Campo, J. M. Rawson and D. A. Haynes, 2017, *Chem. Commun.*, **53**, 11310.

6. D. A. Haynes, L. J. van Laeren and O. Q. Munro, 2017, *J. Am. Chem. Soc.*, **139**, 14620.

Microelectron diffraction implementation at the CNB cryoelectron microscopy facility

Javier Collado-Ávila, Jonathan Piccirillo, José Javier Conesa, David Delgado-Gestoso, Noelia Zamarreño, Teresa Bueno-Carrasco, Francisco J. Chichón, Rocío Arranz-Ávila, José M. Valpuesta, César Santiago

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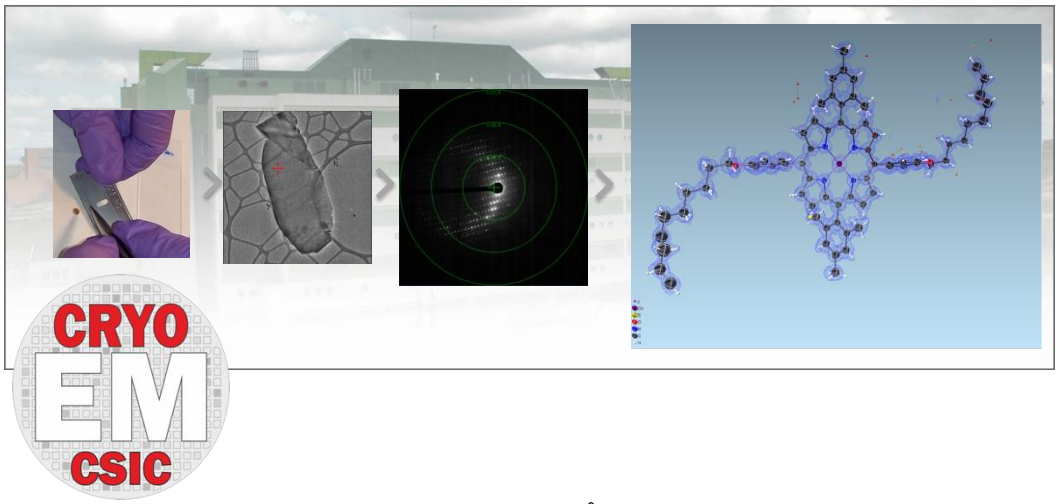
Cryoelectron Microscopy (Cryo-EM) has emerged as one of the most powerful tools in the field of structure biology since the advent of the resolution revolution in the field. New software on image analysis and specially the new hardware used for data collection has positioned the technique at the front of the techniques in molecule structure resolution. A variant of Cryo-EM is the microelectron (MicroED) diffraction of nano crystals, in which the transmission electron microscope is used to diffract crystals of the complete range of molecules that could be crystallized: from small compounds to macromolecules. The flexibility and power of this technique allows us to solve the structure of the crystalized molecules with almost no sample used and in a very short period of time.

We have implemented a robust protocol for MicroED at CNB's CryoEM facility that allows us to solve the structure of a varied range of samples from different users. We are convinced that the established workflow would be of great help to assist researchers from a wide range of scientific concerns, in solving molecules crucial in their investigations.



Scheme or Figure 1: Workflow for the structure resolution at 0.6 Å of a small compound using microelectron diffraction.

Acknowledgements

This research work was also funded by the European Commission – NextGenerationEU (Regulation EU 2020/2094), through CSIC's Global Health Platform (PTI Salud Global)

ORAL PRESENTATIONS

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OC1

Structural and functional insights into hydrogen sulfide homeostasis in pathogenic bacteria

Sofia Soares Costa[1,§], Brenna J. Walsh[2,§], David P. Giedroc[2,*] and José A. Brito[1,*]

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Hydrogen sulfide (H_2S) is an ancient molecule present in Earth's primordial atmosphere and organisms from all Domains of Life soon evolved to utilize it in their physiology. However, H_2S can have either beneficial or toxic effects, depending on the concentration. Therefore, tight regulation of intracellular H_2S and H_2S-derived more oxidized reactive sulfur species (RSS) is paramount for survival of all organisms. [1]

In bacterial pathogens, H_2S/RSS is regarded as an important component in microbial defense mechanisms against oxidative [2] and antibiotic stress, with recent studies highlighting a correlation of endogenous H_2S and antibiotic resistance in *Staphylococcus aureus* and *Bacillus anthracis*. [3] Moreover, H_2S has been shown to revert intrinsic cephalosporin resistance in *Enterococcus faecalis*. [4]

The *cst* (copper-sensing operon repressor-like sulfurtransferase) operon in *S. aureus* encodes a nearly complete mitochondrial-like H_2S oxidation system (S^{2-} to thiosulfate, $S_2O_3^{2-}$). In addition, a *cst*-like operon has also been described in the human pathogen *E. faecalis*. Three enzymes encoded by these two operons include the *E. faecalis* coenzyme A persulfide reductase CoAPR, and the *staphylococcal* multidomain persulfide dioxygenase-sulfurtransferase fusion protein CstB and the sulfide:quinone oxidoreductase SQR, which collectively protect the organism against H_2S and RSS toxicity. [1]

In this work, we describe the X-ray crystallographic structures of full-length *Sa*CstB (native and single cysteine substitution mutants), and the CoA-bound crystal structure of *Ef*CoAPR, to 2.69 Å and 2.05 Å resolution, respectively. Companion cryo-EM data on these enzymes suggest a high mobility of the C-terminal rhodanese domains that may be important for catalysis. The structures of sulfite-bound mutant CstBs suggests a mechanism by which the C-terminal domain facilitates the concerted oxidation of a thiol persulfide (RSSH) to thiosulfate $S_2O_3^{2-}$ and thiol, without the release of the toxic sulfite (SO_3^{2-}) intermediate.

These studies provide an enhanced understanding of the mechanisms of H_2S/RSS homeostasis encoded by the RSS-regulated *cst* operons in bacteria. Moreover, it might serve as foundational work for fragment-based screening in structure-based drug design/repurposing for these enzymes to tackle H_2S-mediated antibiotic resistance in pathogenic bacteria.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857203, and FCT - Fundação para a Ciência e a Tecnologia, I.P., grant through MOSTMICRO-ITQB R&D Unit (UIDB/04612/2020, UIDP/04612/2020) and LS4FUTURE Associated Laboratory (LA/P/0087/2020). SSC and JAB were supported by FCT - Fundação para a Ciência e a Tecnologia, I.P., through 008/BI/2022 and by the framework of Article 23 of Decree-Law No. 57/2017 of August 29, respectively.

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OC2

Flexible, decorated and tight - a new breed of natural anticoagulants

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The hematophagous lifestyle of many invertebrates relies on a complex molecular arsenal that targets the hemostatic, inflammatory and immune responses of their hosts.[1] The central role played by thrombin in the hemostatic system, makes it a prime target for many of these natural anticoagulants.[2] Although many thrombin-targeting molecules use structural modules common to other proteinase inhibitors,[3] evidence has accumulated for the existence of a group of non-canonical, cysteine-less and flexible thrombin inhibitors, distributed across a wide range of evolutionarily distant organisms. We have found that despite their seemingly independent origin, common features of most of these inhibitors are a bidentate binding mode and the posttranslational installation of a potency enhancing *O*-sulfate group on specific tyrosine residues (Figure 1). Using advanced synthetic chemistry methods, in combination with biochemical and biophysical approaches, we unveiled the molecular basis for the specific recognition and inhibition of thrombin by this heterogeneous and unique group of anticoagulants.[4]

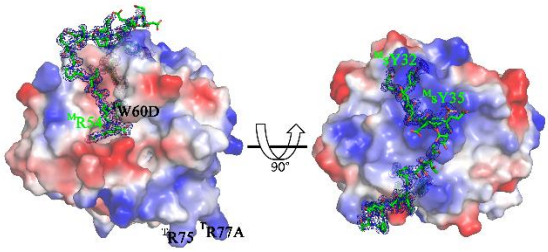

Figure 1: Bidentate binding mode of the tick-derived thrombin inhibitor madanin, with specific recognition of the exosite II of the enzyme mediated by *O*-sulfate tyrosine residues (adapted from Thompson *et al.* (2017) *Nat. Chem.* **9**, 909)

Acknowledgements

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OC3

Base Excision Repair (BER) in the extremely radiation resistant bacterium *D. radiodurans* – a crystallographic view

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D. radiodurans is a pigmented pink/orange bacteria which was first identified in 1956 in canned meat sterilized by ionizing radiation [1]. The bacteria exhibits an extreme resistance to radiation and desiccation and tolerates radiation doses up to 5,000 Grays (Gy) without loss of viability [2]. Despite access to the genome sequence, and decades of investigation, the mechanism of this resistance is still not known. The current hypothesis is that it is related to a compact genome packing, unusual cell membrane, high levels of intracellular Manganese (Mn) and an efficient DNA repair machinery.

We have studied the DNA repair mechanisms of this unusual phenotype for more than fifteen years, and our focus has been on the Base Excision Repair (BER) pathway. The genome sequence of *D. radiodurans* demonstrated an elevated number of BER enzymes compared to other bacteria [3], thus suggesting that this multistep pathway holds an important role for the extremophilic properties of this organism.

In order to address this hypothesis we initially performed structure/function studies of DNA glycosylases (UNG, MUG, AlkA and EndoIII), which initiate the BER process_ENREF_15. In summary our findings demonstrated that they all have undergone structural and/or catalytic modifications which will increase the ability of *D. radiodurans* to maintain the stability of its genome upon exposure to genotoxic stress [4]. More detailed studies of the three isoforms of Endonuclease III (EndoIII1, 2 and 3) demonstrated that they all possess dissimilar properties, indicating that they serve different roles under stress conditions [5].

Recently, mutational studies revealed importance of unique amino acid substitutions in DNA interacting loops in EndoIII1 and novel insight into the role of the FeS cluster of these enzymes[6]. We have also produced and characterized (biochemically and structurally) the two repair synthesis proteins in the last step of BER, DNA polymerase I and DNA ligase A [7–9].

Here we will present an overview of the crystal structures that have been determined of the BER enzymes from *D. radiodurans,* with a special focus on our most recent findings. We will elaborate on how the structures have contributed to an increased understanding of DNA repair generally and under extreme conditions specifically.

Acknowledgements

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OC4

Ar-BIAN-Zinc-based complexes: synthesis, structural characterization, and application as catalysts for cyclic carbonates formation

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Carbon dioxide, CO_2, is the most abundant greenhouse gas emitted as a result of several industrial processes.[1] Carbon dioxide can, in fact, be used as a raw material in the production of a wide variety of industrial products and applications, such as cyclic organic carbonates, apart from the advantage of reducing these existing greenhouse emissions. The process consists of the catalyzed cycloaddition reaction of CO_2 to cyclic ethers, such as epoxides, in a 100% atom economy reaction.[2]

Herein, new neutral [Zn(Ar-BIAN)Cl_2] complexes (where Ar-BIAN = bis(aryl-imino)acenaphthene) were synthesized using a green synthetic methodology, and characterized by elemental analysis, ESI-LS mass spectrometry, FTIR-ATR, and/or multinuclear NMR spectroscopy and, when possible, single crystal X-ray diffraction (Figure 1).

The obtained [Zn(Ar-BIAN)Cl_2] complexes were tested as catalysts in the cycloaddition reaction of CO_2 and epoxides, at the temperature of 333 K, pressures up to 6 MPa, and using various concentrations of catalyst and/or co-catalyst (tetrabutylammonium bromide), under solvent-free conditions.

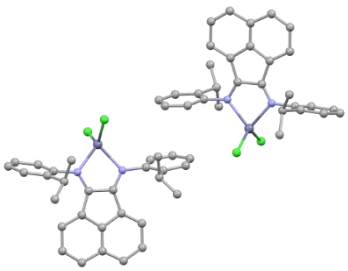

Figure 1: Molecular structure of complex [Zn(2-iPr-C_6H_4-BIAN)Cl_2], displaying two independent isomers in the asymmetric unit.

Acknowledgements

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OC5

Supramolecular structures of camphor sulfonimines

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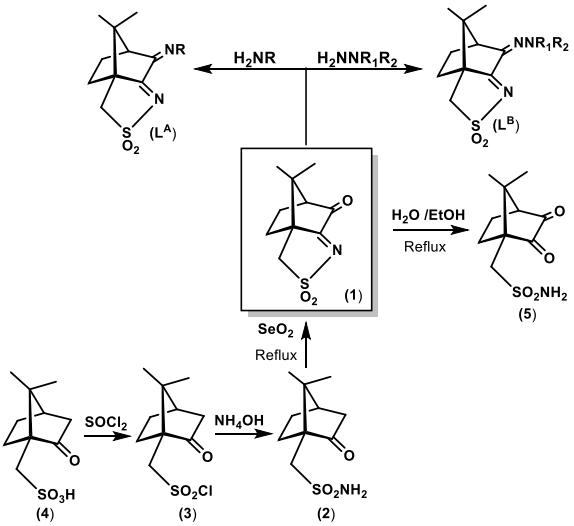
3-oxo-camphorsulfonimide (**1**) is a key compound to synthesize a series a camphor sulfonimines (L^A, L^B, Scheme bellow), used as ligands to obtain complexes for biological applications as antimicrobials or cytotoxic agents or as catalysts, depending on the characteristics of the metal site. 3-oxo-camphorsulfonimide (**1**) is obtained from camphorsulfonic acid (**4**) through three sequential steps that involve acid to amine transformation (**4→2**) followed by amine and ketone condensation, ended by oxidation with selenium and concomitant ring closure (Scheme on the left).



The crystal structures of **1** (tricyclic) and **3** (bicyclic) will be discussed, in particular their supramolecular structures formed by (or absence of) intermolecular interactions.

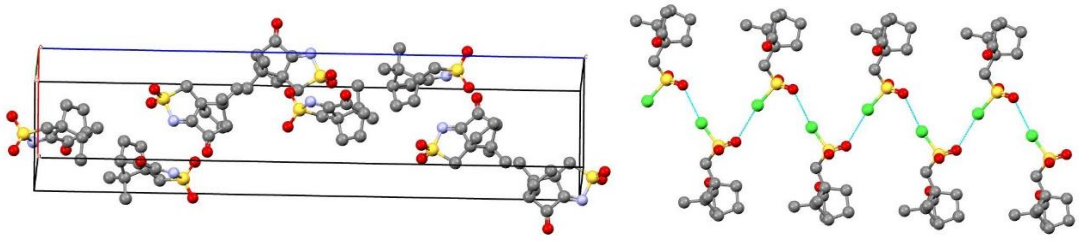


Figure 1. Structural arrangement of **1** (left) and **3** (right)

Acknowledgments

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OC6

Enhancing Metal-Organic Frameworks by Post-Synthetic Modification

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Research on Metal-Organic Frameworks is currently driven towards the need to employ such materials in technological areas. Our research efforts are currently focused on the design of networks based on polyphosphonic acid ligands and rare-earth cations envisaging energy applications.(1) This work presents our most recent results in designing better-performing MOFs that take advantage of proton/ionic mobility and/or exchange. We were able to improve the conductivity and gas sorption of a porous MOF by a simple incorporation of a cation in the channels: the incorporation of K^+ into the pores of [Ln(H_3pptd)]·*x*Solvent (H_6pptd = (5'-(4-phenylphosphonic acid)-[1,1':3',1''-terphenyl]-4,4''-diyl)diphosphonic acid) (Fig. 1) led to a considerable increase in conductivity up two orders of magnitude, reaching 2.1×10^{-2} S cm^{-1} at 40 ºC and 20% (RH) and 0.19 S cm^{-1} at 94 °C and 98% RH.(2) In a similar way, this post-synthetically modified material showed a considerably boost in the adsorption capacity towards CO_2 sequestration as well as being capable to separate C_2H_2 from CO_2 in a complex ternary gas mixture composed of CH_4, CO_2 and C_2H_2.

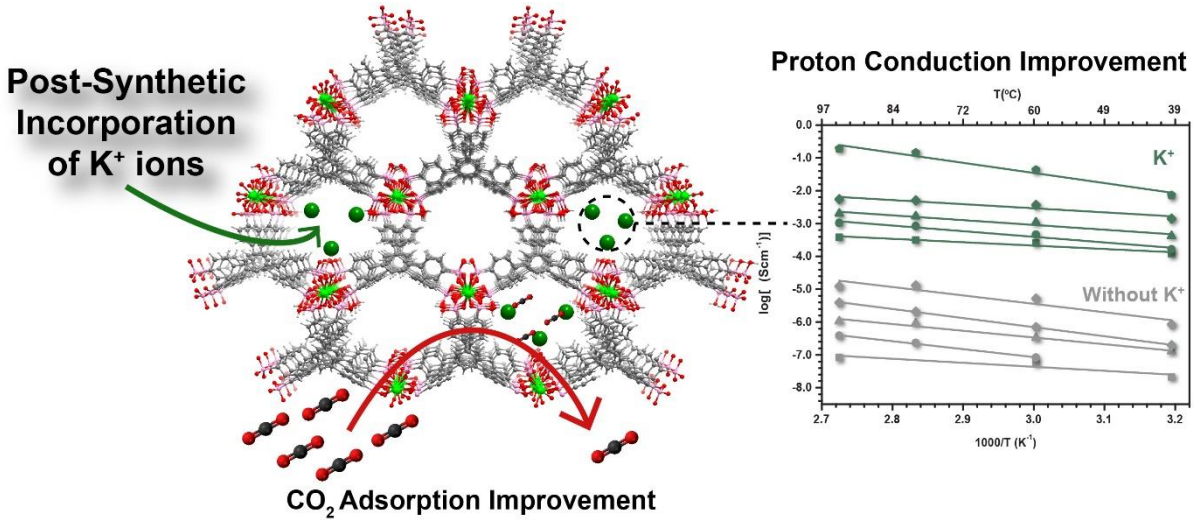

Fig. 1 – Schematic representation of post-synthetically modified [Ln(H_3pptd)]·*x*Solvent by incorporation of K^+ cations resulting in the improvement of both conductivity and gas adsorption properties.

This work was developed within the scope of the project CICECO - Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). RFM gratefully acknowledges FCT for a Junior Research Position (CEECIND/00553/2017). The authors acknowledge the contribution of Sérgio M. F. Vilela, Jorge A. R. Navarro, Paula Barbosa, Germán Pérez-Sánchez, Harriott Nowell, Duarte Ananias, Filipe Figueiredo, José R. B. Gomes and João P. C. Tomé for their collaboration.

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OC7

Arsenite oxidase – a complex enzyme to tackle a complex environmental problem: arsenite water contamination

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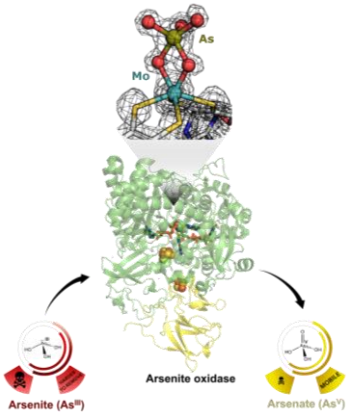
Due to an intricate network of anthropogenic and natural causes, arsenic water-contamination, presents a global threat both to the environment and to public health. A recent study published in Science[1] identified that up to 220 million people are potentially at risk of As exposure, due to groundwater consumption that exceeds the recommended WHO maximum value of 10 µg/L[2]. The enzyme arsenite oxidase (Aio)[3-5] is a good candidate to be used as a biosensor and in remediation, suppressing the current needs, as a solution simultaneously effective, clean, and economically sustainable[6].

The most extensively studied Aio's belong to *Rhizobium* sp. NT-26 (NT-26 Aio) and *Alcaligenes faecalis* (*Af* Aio), and so far, are the only X-ray structures available (PDB IDs: 4AAY, 5NQD and 1G8K[3-5]). These models correspond to highly similar structures of the ligand-free form. Both Aio enzymes contain a large subunit (AioA), that harbors a molybdenum center and a [3Fe-4S] cluster, and a small subunit (AioB), that possess a Rieske [2Fe-2S] cluster and have been shown to oxidize arsenite (As^{III}), as well as antimonite (Sb^{III}) – another known toxic metalloid that functions as a substrate analogue - into the easier to remove and less toxic arsenate (As^{V}) and antimonate (Sb^{V}), respectively[7] (Figure 1).

In our work, optimization of Aio crystallization and soaking conditions allowed us to obtain 4 distinct structures, with different forms of Aio-reaction intermediates, that diffracted up to *ca* 1.5 Å resolution. The structures show reaction intermediates of Sb/As oxyanions bound to the active site, with µ-oxo bridges binding Sb/As to the Mo atom. Analysis of bond lengths and geometry of the ligands at the vicinity of the active center allowed us to revisit the catalytic mechanism of As oxidation.

Comprehending the biophysical and structural insights of arsenite oxidases will contribute not only to elucidate the reaction mechanism of these unique enzymes, but also to develop new biotechnological applications in As/Sb contaminated-water treatments.



Scheme or Figure 1: Representative scheme of arsenite oxidase catalysing the oxidation of arsenite - very toxic and hard to remove - into the less harmful and less mobile arsenate.

Acknowledgements

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OC8

Archaeometric Study of Chalcolithic Ceramics from Lisbon

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The Chalcolithic in the Lisbon region (3rd millennium BC) is mainly characterized by an economic intensification and specialization in the production field with the introduction of new technologies, namely metallurgy, of which the building up of powerful defenses on the habitat sites can be a repercussion. The Chalcolithic period in this region is usually divided into three phases, with a chronological meaning, to which specific types of ceramic decoration are associated. Thus, Early Chalcolithic is characterized by cylindrical cups with polished corrugated outer surface, Full Chalcolithic by the so-called acacia-leaf decoration, and Late Chalcolithic by the Bell Beaker pottery. The aim of our research is to characterize all these kinds of ceramics trying, at the same time, to identify sources of the clay used in the pottery manufacture. To do so, a total of about 150 ceramic shards recovered at four Chalcolithic settlements (Vila Nova de São Pedro, Penedo do Lexim, Espargueira and Baútas) were sampled and analysed at textural, chemical and mineralogical level. A total of 19 samples of clay deposits collected in areas of influence of each of the archaeological sites under study were also analysed.

Textural analysis using Optical Microscopy of cross sections allowed the characterization of pastes and inclusions. Chemical characterization was undertaken using Micro Energy Dispersive X-ray Fluorescence Spectrometry in powder pellets. Elements Si, Al, Fe, Ca and K were identified and quantified as major elements, Ti and Mn as minor, and Ce, Sr, Zn, Cr, Rb, Co and Th as trace elements. Mineralogical characterization was undertaken using X-Ray Powder Diffraction to analyze ceramic pastes, allowing the identification of quartz, phyllosilicates, potassium feldspars and plagioclases, iron oxyhydroxides and mafic minerals as the main phases, while calcite was identified only in few samples. Petrographic Microscopy in thin sections and Raman Microspectroscopy was used for identification of non-plastic inclusions.

Results suggest that production techniques may have remained similar throughout all the Chalcolithic period, having been used the coiling technique for larger vessels and the ball shaping technique for smaller vessels, and firing temperatures between 700 and 800 ºC. Multivariate analysis of chemical and mineral contents of analysed ceramics suggests that multiple sources of raw material must have been used in the manufacture of the pottery collected at the four Chalcolithic settlements.

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OC9

Energy landscape and piezoelectric properties of diphenylalanine nanostructures

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Piezoelectric properties of crystalline organic and bioorganic materials are determined by the dipole moments of individual molecules and their spatial packing. The role of vectored forces on the packing of small aromatic peptides and the correlation between their structure and properties have been studied recently.[1,2] At the same time, molecular packing is closely related to the conformation of the molecules, which also determines their polar properties. Though most biomolecules possess a nonzero dipole moment this moment can be significantly reduced in a crystal due to an improper molecular conformation.

In this work, we studied the relationships between the conformation and polar properties of an individual molecule of diphenylalanine (FF) dipeptide and the piezoelectric properties of various nanostructures they form. We systematically analyzed crystallographic data available in Cambridge Structural Database[3] and revealed three molecular conformations typical for FF crystalline structures: *cis*-, *trans*-, and *linear*-. By means of quantum chemical calculations, we constructed an energy landscape and a map of dipole moments for an individual FF molecule and compared them with molecular configurations from crystallographic data. We found that aromatic interaction between phenyl rings in the molecule makes *cis*- configurations, in general, less stable than *trans*-ones. However, in a few specific cases corresponding to local energy minima, *cis*- configurations remain stable and form well-known nanotubes.[4]

On the other hand, dipole moments of *trans*-configurations exceed those of *cis*- and *linear*-. We found that the primary source of the dipole moment of FF is zwitterionic charges, whereas aromatic interactions between phenyl rings and the length of the side branches do not influence that. Though *trans*-FF molecules tend to form non-polar centrosymmetric crystals, these crystals possess layered crystal structure with individual layers demonstrating pronounced out-of-plain polarization and, thus, piezoelectric properties. These crystals can be chemically exfoliated to get individual layers representing 2D piezoelectric nanomaterials suitable for various electromechanical applications.

The *linear*- configurations still require a detailed study. However, preliminary analysis showed that the molecules with longer side branches tend to form less ordered crystal structures similar to polymers. Such disordered crystals possess small dipole moments with weak piezoelectric properties. The obtained results uncover the role of molecular conformation on the functional properties of molecular crystals and provide additional functionality for the design of organic piezoelectrics.

Acknowledgements

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A XANES approach to the blue pigments in ceramic heritage

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The blue colour has been extensively used as a ceramic pigment, particularly in Chinese porcelain production. Cobalt blue is a stable pigment at high temperatures and seems to be a little affected by the firing atmosphere, unlike other transition metals, which change their colour depending on the firing atmosphere. Synchrotron radiation-based techniques have been applied to studying cobalt speciation in the Chinese blue-and-white porcelain to determine the cobalt and iron oxidation state and local coordination environment. The relationship between the firing atmosphere conditions and cobalt speciation has not been studied yet. In this work, underglaze blue models were produced and fired in air or under reducing atmosphere to ascertain the effect of the firing atmosphere on cobalt and iron speciation. The experimental results were compared with data obtained on historical samples of ancient porcelain shards. The microstructure and colour of the underglaze blue models were ascertained by variable pressure scanning electron microscopy (VP-SEM-EDS), using a hyperspectral imaging camera (Vis-SWIR reflectance spectroscopy) and colorimetry. The formal valence and coordination of cobalt and iron ions of the glaze were determined by X-ray absorption spectroscopy (XAS), particularly X-ray absorption near edge structure (XANES), using synchrotron radiation. Spectral features demonstrated that both cobalt and iron speciation were affected by the firing atmosphere and therefore could be used to ascertain the firing atmosphere.

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OC11

Jumping from crystalline to amorphous drugs: How can we understand their molecular-level organization?

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The conversion from crystalline into amorphous forms offers one the best solutions for active pharmaceutical ingredient (API) solubility problems. The amorphous forms of an API represent a higher energy state, which often coincides with an increase in solubility and bioavailability.[1, 2]

Several studies indicate that the production methods and storage conditions have a notable influence on the amorphous product and its physicochemical properties. Examples are the anti-inflammatory drug indomethacin and the beta-lactam antibiotics, where different stability, dissolution and pharmaceutical performances were observed for amorphous forms prepared *via* different methods.[3] These reports raise a fundamental question regarding the amorphous state: ***How can an amorphous form of a defined chemical compound differ from another amorphous form of the same compound***? This question can be answered for crystalline materials, as different crystal structures of solids give rise to different polymorphic forms with unique characteristics.[4] In disordered amorphous materials, no long-range order can give rise to a phenomenon like this, yet differently prepared amorphous forms do exhibit different physicochemical properties. It becomes clear that differences in the physicochemical behavior between distinct amorphous forms may be fundamentally associated with the intricacies of their local molecular arrangements.

Although the identification, characterization and quantification of amorphous APIs has received considerable attention, little is known about their local ordering.[5] Conventional and routine structural characterization methods, such as powder X-ray diffraction (PXRD), become featureless in these cases.[6] However, information extracted from the PXRD data can still be used by applying the pair distribution function (PDF). Used as a fingerprint to identify both crystalline and amorphous organic materials, the applicability of PDF in structure determination is still limited. Two reasons are: i) weakly diffracting atomic species of organic materials; and ii) lack of implementation of practical rigid-block molecular description in PDF refinement programs.[6] Herein we show how the combination of PDF with other solid-state characterization techniques and molecular dynamics, can give important information about the local structure of different amorphous phases of hydrochlorothiazide (HCT), prepared *via* different methods.

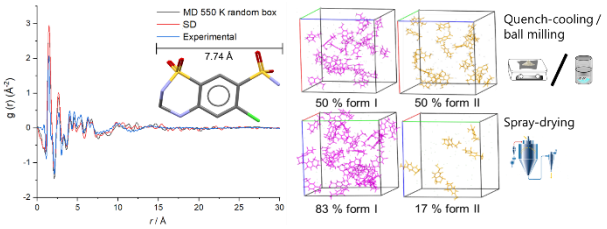

Figure 1: Experimental and simulated PDF data obtained for amorphous HCT (left). Dihedral population distribution of amorphous HCT when prepared *via* quench-cooling / ball milling or spray-drying methods (right).

Acknowledgements

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OC12

Formate dehydrogenase presents a retention cavity in the substrate tunnel

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Mo- and W-formate dehydrogenases (Fdhs) are unique prokaryote enzymes that catalyze the reversible reduction of CO_2 to formate. The chemical transformation of this greenhouse gas, into useful products, becomes increasingly important as its atmosphere levels continue to rise because of human activity. We aim to better understand the bidirectional catalytic mechanism of these enzymes in order to bioengineer these biosystems towards large scale CO_2 reduction. Recent studies in a new highly active and O_2 stable W-Fdh from *D. vulgaris*[1], proposed a second substrate tunnel to convey CO_2 molecules to the active site independently of the main Formate Tunnel. In this work we attempted to validate the existence of this proposed tunnel, by performing Soak-and-Freeze[2] experiments at ESRF's High Pressure Freezing Laboratory with Kr and O_2. Our results showed no gas molecules in the proposed CO_2 Tunnel, because either the tunnel is not present, or its internal radius was reduced by the increased pressure. Nonetheless, our results showed two Kr and two O_2 in a cavity attached to the Formate Tunnel and close to the active site. These findings led us to propose a Retention Cavity (Figure 1), that would transiently hold substrate molecules to be conveyed to the active site, thus increasing the enzyme's catalytic efficiency. Further biochemical and structural studies will be performed in order to validate the existence of this Retention Cavity and ascertain the exact nature and catalytic role of this interesting structural feature.

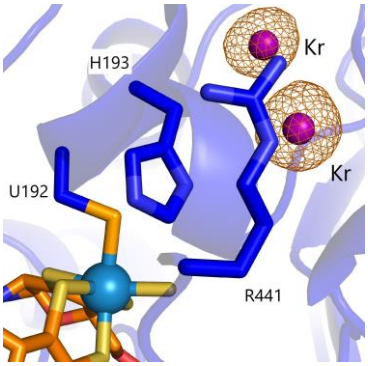

Figure 1: *D. vulgaris* FdhAB subjected to the Soak-and-freeze method with 200 bar of Kr (in blue), displaying two Kr atoms (in violet) modelled in the proposed Retention Cavity, and their respective 2Fo-Fc electron density maps at 1σ (in orange). Additionally, the W atom (in grey) and its coordination sphere are shown. Images generated with PyMOL.

Acknowledgements

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POSTERS

Molecular details of the dual function of Dps2, a nano-cage protein from Deinococcus radiodurans

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Deinococcus radiodurans is to date the most resistance organism to ionizing radiation and UV radiation. Its success has been attributed to its effective response in protecting its proteome enabling this organism to detoxify reactive oxygen species (ROS) as well as fully repair its integral genome1. Interestingly, high intracellular manganese has been positively correlated with its high resistance to radiation1. D. radiodurans encodes two DNA binding proteins under starved conditions (Dps), Dps1 and Dps2 which are main players in metal homeostasis, namely in the iron and manganese storage2-4. Our main goal is focus on the structural characterization of Dps2 to unveil its mechanism of metal homeostasis as well as DNA interaction. Dps2 presents an interesting large N-terminal (40 residues length without the signal peptide), however due to its high flexibility confirmed by our previously results5, its structure is yet to be resolved. To solve the full-length structure, Dps2 was expressed, purified and followed by crystallization. The best diffracting crystals were obtained at 2.8 Å resolution, and the structure revealed additional features in the extended N-terminal tail. Moreover, the structure of the Dps2-DNA complex has been address by Cryo-Electron Microscopy Single Particle Analysis. We access the Microscopy Unit at University of Helsinki through INSTRUCT and IMpaCT to collect a dataset on a Talos Artica 200kV microscope of Dps2-DNA complex. However the resolution of this dataset was very low, so we have recently collected a high resolution dataset in the 300 kV Titan Krios at CM01-ESRF, this data is currently under processing.

Acknowledgements
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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857203- IMpaCT.This work benefited from access to the Electron Microscopy Unit-University of Helsinki, Finland, Instruct-ERIC centre. Financial support was provided by Instruct-ERIC Access proposal: PID- PID-12696.The Titan Krios dataset was collected at the CM01, ESRF as part of the Iberian BAG MX2269. We are grateful to the Local Contact*, Dr. Eaazhisai Kandia*h at the ESRF for providing assistance in using beamline CM01.

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Structural analysis of tree distinct classes of Flavodiiron proteins obtain by *X-ray Crystallography* and *Single-Particle Cryo-EM*

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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.

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]. This protein is a prototype of modular enzymes that may harbor up to three additional domains, with putative extra redox centers for example rubredoxins, flavins, Fe/S clusters, besides the two common core domains[2].

The aim of this study was to determine the structure of the three classes (A, B and F) of enzyme Flavodiiron protein (FDP) to understand the way of its response to its substrates (O_2, NO and H_2O_2) at a structural level.

To obtain this structural information, different structural analysis were carried out, namely Small-Angle X-ray Scattering, X-ray crystallography and Single Particle Cryo-EM. At this point, it has been possible to obtain crystals of the two diffracted enzymes (classA and B) with the maximum resolution obtained for class A was 2.5Å and for class B was 2.8Å and obtain the data for class F by Single particle collected in Titan Krios.

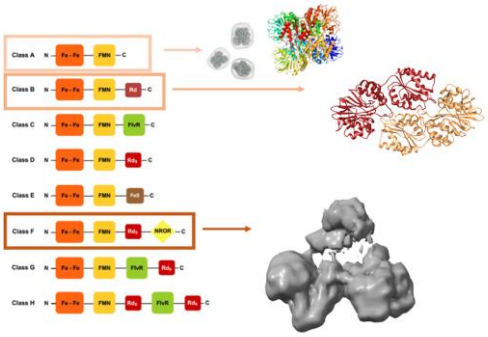

Figure 1: Schematic representation of the several Classes of FDPs

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Structural details for mucin-type O-glycan recognition by *Bacteroides thetaiotaomicron*

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Abstract:

The human gut houses a dense population of commensal bacteria that lives in close contact with the host, being involved in several biological processes[1]. One of the most prominent gut commensals, *Bacteroides thetaiotaomicron*, holds a high number of gene clusters designated Polysaccharide Utilization Loci (PULs)[2]. These clusters, encode a set of surface proteins that act in concert to target, adhere and degrade different glycans as sources of nutrients. These include degrading enzymes, adhesive Carbohydrate-Binding Modules (CBMs), SusD-type and lectin-type domains[3]. In the absence of dietary glycans, *B. thetaiotaomicron* has the ability to degrade mucus *O*-glycans that protect the gut epithelium and this shift can damage the colonic mucus barrier and promote pathogen susceptibility[4]. Our aim is to uncover and characterize proteins that are involved in targeting and degrading host *O*-glycans to understand how this bacterium thrives in the gut. Our integrative approach combines glycan microarray technology with X-ray Crystallography and biophysical methods[5] to uncover CBMs and SusD proteins of a *B. thetaiotaomicron* PUL directed at mucin-type O-glycans. The glycan microarray analysis identified a family 32 CBM that exhibited a sharp specificity to core 1 (Galβ1-3GalNAcα-Thr) and core 2 (GlcNAcβ1-6[Galβ1-3]GalNAcα-Thr) *O*-glycans. The bound structures of CBM32 revealed a pocket

with a canonical galactose-binding site. Specificity for core 1 and core 2 O-glycans is conferred by a direct hydrogen bond between an Aspartate and the GalNAc glycan moiety. The SusD-like protein structure was solved at 1.4 Å resolution and efforts are ongoing to discover their ligands. The integration of the structural data with functional studies will be crucial to decipher human-microbe communication and for the development of innovative pharmaceutical solutions.

Keywords: Human gut microbiome, *Bacteroides thetaiotaomicron*, mucin *O*-glycan recognition

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P4

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

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Structural insights on the glycan-binding function of a novel protein from *Bacteroides caccae*, a commensal bacterium from the human gut

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The human gastrointestinal tract harbors a diverse community of commensal bacteria, named gut microbiota. These microorganisms are beneficial to the human gut, due to breaking down dietary polysaccharides, such as starch, which cannot be digested. However, in a low-fiber diet, host carbohydrates provided by the intestinal mucus, which is mainly composed of *O*-glycosylated glycoproteins, 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[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* (*B. caccae*) has been reported to express different polysaccharide utilization loci (PULs), which encode for all the genes necessary for the breakdown and uptake of carbohydrates, such as those that code for glycoside hydrolases (GHs), M60-like metallopeptidases (Pept_MA) and their appended non-catalytic ancillary carbohydrate-binding module of family 32 (CBM32)[2]. Herein, we report the structure of BC16100-C (a member of the CBM32 family of a Pept_MA from the *B. caccae* strain), solved using X-ray crystallography methods (Figure 1). The 3D structure reveals insights on the putative amino-acid residues that recognize the carbohydrate ligands. Attempts to produce the complex structure with ligands identified by glycan microarray analysis are on-going.

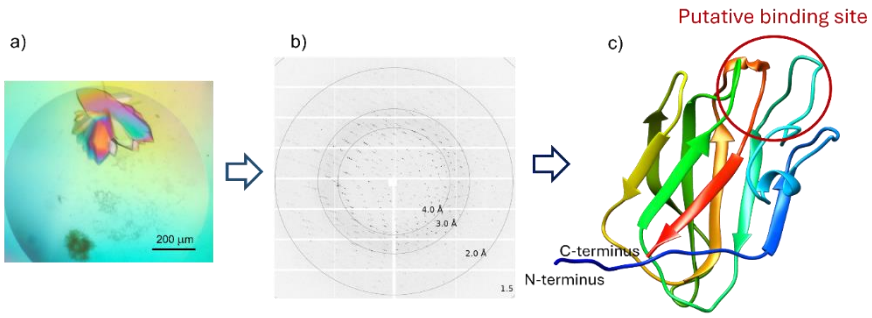

Figure 1: Simplified representation of the steps followed to solve the BC16100-C structure by X-ray crystallography. a) Crystals formed of BC16100-C viewed under polarized light. b) X-ray diffraction image of BC16100-C, collected from a synchrotron source (14keV, 0.05s exposure, 1º phi rotation. c) Ribbon representation of BC16100-C, colored from the N-terminus to the C-terminus. The putative binding site is indicated by the red circle.

Acknowledgements

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Structure-based virtual screen for potential AftD inhibitors, using a drug repurposing strategy

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Tuberculosis (TB) is one of the leading causes of death worldwide and is a disease caused by the infection of *Mycobacterium tuberculosis* (Mtb). The emergence of multidrug-resistant mycobacteria has prompted the need to better understand these mechanisms and develop novel therapeutics[1].

Arabinofuranosyltransferases (AraTs) are membrane enzymes involved in the biosynthesis of arabinan, an important component of the unique cell-envelope of Mtb. High virulence and resistance of Mtb to common antibiotics is related to the protective role of the mycobacterial cell wall, which means that these enzymes are attractive targets against TB.[2–7]. This family of proteins, essential for bacterial survival and growth, can be divided into two subgroups: Embs (EmbA-C) which are proteins known as targets for ethambutol, a first-line drug for TB treatment; and Afts (AftA-D) which are not inhibited by any known drug, thus being new potential drug targets against TB.

In order to overcome the existing multidrug-resistant bacteria, in this work we performed a structure-based virtual screening with AutoDock Vina to identify molecules with potential to inhibit AftD, a protein whose 3D structure has already been determined by our group (AftD from *M. abscessus* at 2.9 Å resolution) using single particle cryo-electron microscopy (Cryo-EM).[8]

We used the RepoDB database (http://apps.chiragjpgroup.org/repoDB/)[9], which comprises a collection of previously approved compounds , allowing us to use a drug repurposing method, a process of discovering, validating, and marketing of previously approved drugs for new applications, which is an approach of great interest to academia and industry, due to reduced time and costs associated with repurposed drugs.

This *in silico* workflow allow us to select the best candidates, as we intend to perform enzymatic, biophysical and structural assays to investigate their interactions with AftD.

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Crystallographic studies on enzymes involved in sulfide removal in the purple sulfur bacterium Thiocapsa roseopersicina

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Sulfur is among the most important chemical elements in human biology, exists in oxidation states from -2 (in H_2S) to +6 (in SO_4^{2-}), and is a critically important constituent of vitamins, hormones and amino acids, therefore playing an important role in specific metabolic processes. Since Earth's primordial times, life was constantly challenged and organisms were compelled to create new strategies to evolve, adapt and survive to the hastily changes in the environment. [1] One of the most significant leaps in evolution was the use of hydrogen sulfide (H_2S), an abundant gas on the early atmosphere derived from the intense volcanic activity, in energy production pathways. [2] *Thiocapsa roseopersicina*, a purple sulfur photosynthetic bacterium, is among the organisms that evolved to use H_2S and other reduced inorganic sulfur compounds (*e.g.*, $S_2O_3^{2-}$, SO_3^{2-}, S^0) in anaerobic photosynthesis to generate reducing equivalents and energy. This bacterium is involved in the regulation of S^{2-} levels in the environment, while performing carbon dioxide fixation in seawater, a process that is essential for the sustainability of life on Earth. [3,4]

The first step in H_2S conversion is mediated by sulfide:quinone oxidoreductases (SQR), membrane-bound flavoproteins that display two interdependent catalytic activities, those of sulfide dehydrogenase and sulfide:quinone oxidoreductase. [5] The sulfide dehydrogenase activity can also be catalyzed by flavocytochrome *c*:sulfide dehydrogenase (Fc*c*) enzymes, located in the periplasm. SQRs directly transfer electrons from sulfide oxidation to higher potential membrane complexes, while Fc*c* enzymes indirectly via cytochrome *c* proteins, which are reduced through their covalently bound heme cofactors. [6] Nevertheless, the mechanisms behind SQR and Fc*c* enzymatic activities in *T. roseopersicina* are still unclear in detail.

Herein, we describe the biochemical characterization and preliminary crystallization studies on a novel type VI SQR (*Tr*SqrF) and the multidomain flavocytochrome *c* (Fc*c*AB) from *T. roseopersicina*. *Tr*SqrF and *Tr*Fc*c*AB structural characterization might provide a deeper understanding of the ancient, anoxic and energy-generation processes in *T. roseopersicina*, [3] revealing a correlation between H_2S and carbon cycles, while elucidating the impact of this organism on Earth's ecosystem sustainability.

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Engineered proteins targeting SARS-CoV-2

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The ongoing COVID-19 pandemic, caused by the SARS-CoV-2 virus, has affected over 500 million people, and resulted in over 6 million deaths worldwide. Despite the success of current vaccines, there is still the need for complementary therapeutic options targeting specific viral elements, for example, the Spike protein responsible for viral engagement and entry into host cells[1,2]. In the present work, antiviral proteins were computationally designed to target the SARS-CoV-2 Spike protein.

Four design proteins (Lead 1, 2, 3 and 4) with a three helical bundle topology were expressed in *Escherichia (E.) coli* and purified from inclusion bodies. The secondary structural elements of the proteins were analysed by far-UV circular dichroism (CD) and their thermal stability by differential scanning fluorometry (DSF). Leads 1 to 4 presented relatively low stability, thus compromising their production yields. Therefore, another design was generated (Lead 5) to present higher stability. Lead 5 was successfully expressed and purified from *E. coli* and analysed by CD. Biolayer interferometry was used to measure binding between Leads 1 to 5 and the viral target. Leads 1 to 4 had greater affinity than Lead 5 despite being less stable.

To support the computational designs, we attempted to structurally characterise the interaction of Leads 1 to 4 with RBD. To achieve this, the Leads were fused to maltose binding protein (MBP) not only to increase stability, but also to be used as a crystallization chaperone. Despite high protein production yields, the ensuing crystallization trials have not yet been successful.

Simultaneously, a nanoparticle displaying multiple copies of Lead 5 was designed with the intention of increasing its inhibitory efficiency. Coarse Grain simulations were firstly used to validate the nanoparticle, followed by E. coli expression and purification. The nanoparticle was imaged using negative staining transmission electron microscopy. With this approach, we hope to develop a new therapeutic platform against SARS-CoV-2.

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Structure of Engineered Protein Targeting Zika Virus Envelope Protein

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Synthetic proteins were computationally engineered to specifically bind to the Zika virus (ZIKV) fusion loop (FL) located on the (E) envelope protein. FL is a highly conserved region among flaviviruses that mediates cell infection and target of neutralising antibodies. These engineered proteins contain the immunogenic region of human neutralising antibodies (NAbs) grafted into selected scaffold proteins, such as cyclophilin, galectin-8 or interleukin.

The calculated binding affinity of the engineered proteins to the FL was used as selection criterium. The best candidate (ZVPA3) was experimentally synthesized. It was shown to bind to its target with high-affinity, and it was able to efficiently neutralise ZIKV in vitro. The protein structure was determined by X-ray crystallography to validate the computational design, as well as to offer additional insights into the structural basis of flaviriral neutralisation targeting the FL envelope protein (Figure 1).

Studies are on-going to produce ZIKV E, NS1 proteins and virus-like particles (VLPs) in mammalian cell lines and form complexes with the synthetic proteins mimicking NAbs. The goal is to elucidate their 3D structures by X-ray Crystallography or single particle analysis cryo-electron microscopy (cryo-EM). understanding of the structural and molecular basis that dictate high affinity interactions between antigens and their respective antibodies and establishing a platform to engineer improved vaccine antigens

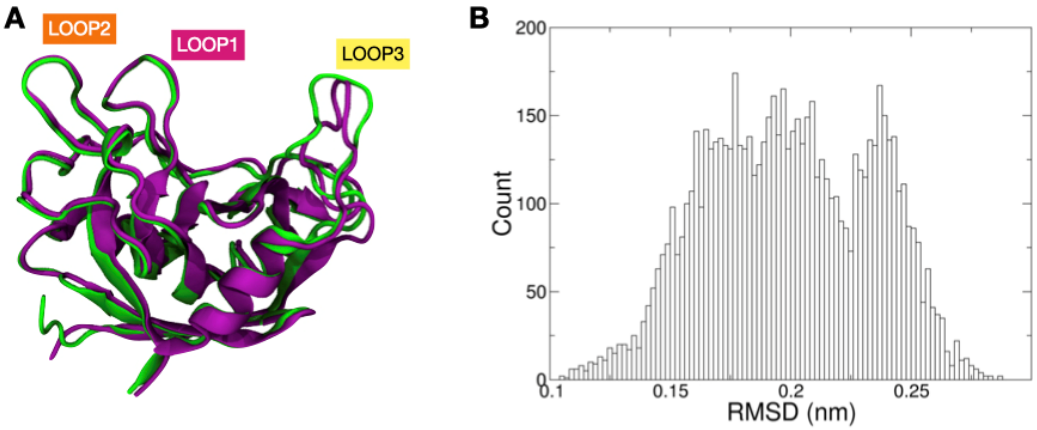

Figure 1: Superposition of crystal structure of ZVPA3 with the predicted computational one (A), RMSD distribution of a trajectory of 100 ns of molecular dynamics using the experimental structure (B)

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Structural studies of mycobacterial arabinofuranosyltransferase AftA: aiming to impair the cell wall synthesis of Mycobacteria

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Tuberculosis (TB) remains one of the world's deadliest communicable diseases, estimated to have killed 1.3 million people in 2020.[1] The organism responsible for TB, *Mycobacterium tuberculosis*, has a unique cell wall structure of composed mainly of mycolic acids, arabinogalactan, peptidoglycan and lipoarabinomannan, that accounts for its unusual low permeability and resistance towards common antibiotics.[2] Arabinofuranosyltransferases (Aft) are membrane proteins involved in the synthesis of essential components of *M. tuberculosis*' cell wall, such as arabinogalactan and lipoarabinomannan.

AftA transfers the first arabinofuranose unit to the galactan core, allowing other transferases to continue the polymerization of arabinan. This first addition of arabinofuranose is essential in *M. smegmatis*, supporting the importance of AftA function and its potential as drug target for the development of novel therapeutics against TB.[3] We aim to determine the 3D structure of AftA to get insight on its biological function and for structure-based drug design.

AftA from *M. vanbaalenii* is a 68 kDa membrane protein, with 66% identity to its *M. tuberculosis* homolog. AftA was expressed in *E. coli*, purified in detergent (DDM) and reconstituted into 1E3D1 nanodiscs. We assembled a protein complex between AftA nanodiscs and a megabody (100kDa) to improve Single Particle Cryo-EM pipeline, and recently collected data on a 200kV FEI Talos Arctica TEM, at the Instruct Centre FI. We were able to build an initial 3D model of the complex, albeit incomplete, since the set of particles of the complex only made up 9.5% of the dataset collected. Further optimizations in sample preparation and extensive data collections will be required for structure determination of the complex.

The 3D structure of AftA will provide insight on the biology of mycobacterial cell wall synthesis and will open the door to develop novel therapeutics against TB.

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THE ROLE OF RuvBL1/2 IN THE ASSEMBLY OF PROTEIN-RNA snoRNP COMPLEXES

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RuvBL1 and RuvBL2 (R1R2) are highly conserved eukaryotic proteins of the AAA+ ATPase family, and closely related to the bacterial ATP-dependent DNA helicase RuvB. They form oligomeric rings, assemble into homo-hexamers, hetero-hexamers and/or hetero-dodecamers, and bear a regulatory Domain (DII) which is unique within the AAA+ ATPase superfamily. R1R2 are key players in transcription, mitosis, apoptosis, and other cellular events. They not only integrate different large molecular complexes such as Ino80 and R2TP, but also ensure the assembly of macromolecular complexes like snoRNPs, namely: the U4-U6-U5 spliceosome, the Box H/ACA snoRNP, the Box C/D snoRNP and the Telomerase complex, making them an attractive drug-target.

The molecular mechanisms behind R1R2's role during snoRNP assembly processes are still unknown. Hence, the aim of this work is to shed light on their early assembly, starting with the translation of crucial protein components like the catalytic active DKC1 in Box H/ACA snoRNPs. After translation DKC1 might interact with SHQ1 which can act as a chaperone and prevent unspecific interaction of DKC1 with RNAs. Subsequently, SHQ1 may guide the complex to the nucleus where the complex is thought to interact with R1R2 to facilitate the maturation of Box H/ACA snoRNP complexes. To characterize and follow the formation of the different complexes we will use size exclusion chromatography, dynamic light scattering, differential scanning fluorimetry and, surface plasmon resonance. After the confirmation of stable complex formation, we aim to determine the 3D structures of these complexes using X-ray crystallography and Cryo-EM.

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Structure and Function of a Dodecameric Machine: The RuvBL1/RuvBL2 and its Role in the Large Macromolecular Complex PAQosome

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RuvBL1 (R1) and RuvBL2 (R2) are highly conserved eukaryotic proteins of the AAA+ ATPases family. They are the core engine of the R2TP complex and were described to participate in other large molecular complexes, such as Ino80 and TIP60.

The R2TP complex – R1, R2, RPAP3, and PIH1D1 – together with the Prefoldin-like complex – URI1, PFDN2, ASDURF, UXT, PFDN6, and PDRG1 – form the PAQosome, which assists HSP90 in the assembly and maturation of different complexes that are essential in many cellular processes. However, the assembly mechanism and how the PAQosome function as a multi-unit chaperone complex remains unclear. In addition, different studies suggest that the PAQosome is also involved in ciliogenesis and in the development of cancer and ciliopathies, making it a potential attractive drug target.

In this study, we aimed at characterizing the different components of the PAQosome by combining functional studies with structural data to better understand the importance of this molecular machine.

TurboID is a novel approach used to study protein localization and interactions in living cells, also allowing the detection of weak and transient interactions with high sensitivity. We applied the methodology to R1/R2 to analyze their interactome and identify interactors in the context of important large cellular complexes. We were able to detect two proteins known to be part of the PAQosome, namely, URI1 and UXT. Their interaction with R1/R2 was confirmed by Surface Plasmon Resonance (SPR), using a recent method developed by our team which allows the kinetic characterization of an interaction regardless of the purity of the immobilized ligand (Extract2Chips). We also optimized the production process of R1/R2/URI1 and R1/R2/UXT ternary complexes and characterized them with other biochemical and biophysical tools. This will hopefully lead to homogeneous and monodisperse samples that will be vital to solve the 3D structures and shed light on such important biological processes.

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Structural analysis of a computationally designed DyP-type peroxidase with improved thermostability

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Dye-decolorizing peroxidases (DyP) are microbial enzymes that can efficiently oxidize a plethora of substrates like anthraquinone and azo dyes, phenolic and nonphenolic lignin units, metals, among others. Therefore, DyPs are interesting candidates for biotechnological purposes. However, DyPs usually lack robust stability properties required for industrial processes.

The *in silico* method PROSS[1] was used to design a variant of the DyP from *Pseudomonas putida* (PpDyP), with increased thermostability. This variant named PpDyP PROSS included 29 mutations, displays an upshift of its optimum temperature from 15-30ºC to 60-70ºC and exhibits a melting temperature of 88ºC, a 25ºC increase from the wild-type (unpublished data). Furthermore, PpDyP PROSS shows a 3-fold higher yield production and 5-fold higher catalytic efficiency (k_{cat}/K_m) for H_2O_2 and ABTS as compared to the wild type.

In this work we will use X-ray crystallography to solve the structure of this thermostable variant, to investigate the role of the mutations suggested by PROSS and unravel the key structural determinants behind the improved thermostability.

PpDyP PROSS orange thin needle-shaped crystals reached maximum dimensions of 10 x 10 x 10 µm. Diffraction data were collected at ALBA Synchrotron Light Facility (Barcelona, Spain) in the BL13-XALOC beamline[2] and were processed with a resolution of 2.3 Å. PpDyP PROSS crystals belong to P $2_12_12_1$ space group with cell dimensions of a = 49.9 Å, b = 84.7 Å, c = 128.2 Å, and two protein molecules per asymmetric unit, corresponding to 43% of solvent content. Structure determination using PpDyP wild-type structure as search model is currently ongoing.

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Improving *Desulfovibrio vulgaris* Hildenborough [NiFeSe] Hydrogenase Resistance to Oxidative Inactivation

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Growing concerns with severe global warming and fossil fuel depletion have stimulated research into alternative and clean energy sources. Biological hydrogen production is a promising route towards a new energy paradigm and a circular economy. Hydrogenases are the enzymes that in nature produce and consume hydrogen. They are very efficient catalysts due to their high turnover rates for H_2 oxidation and production, but they are susceptible to inactivation by O_2.

In the structure of the [NiFeSe] hydrogenase from *D. vulgaris* Hildenborough, exposure to O_2 leads to the irreversible oxidation of Cys75 to sulphinate [1,2] A hydrophilic water channel leading to the active site was identified as the likely oxidation route[3]. This channel has a branch lined with hydrophobic residues unique to this enzyme. To hinder O_2 access to Cys75 along this channel system, residues Gly50 and Gly491 in the large subunit were mutated to Thr, and Ala or Ser, respectively. The G50T mutation is located near the end of the hydrophilic branch and had no significant effect. However, both the G491A and G491S mutations, located before the branching point, significantly protected Cys75 against irreversible oxidation.

We aim to block the hydrophobic branch of the channel, near the junction with the hydrophilic channel bay creating variants in order to restrict O_2 access to the active site with the least possible impact on the enzyme activity. Here, we report the production and characterization of the variants G17E in the small subunit and G50T(large subunit)G17E(small subunit).

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Polytypism in Nanostructured $AgBiSe_2$

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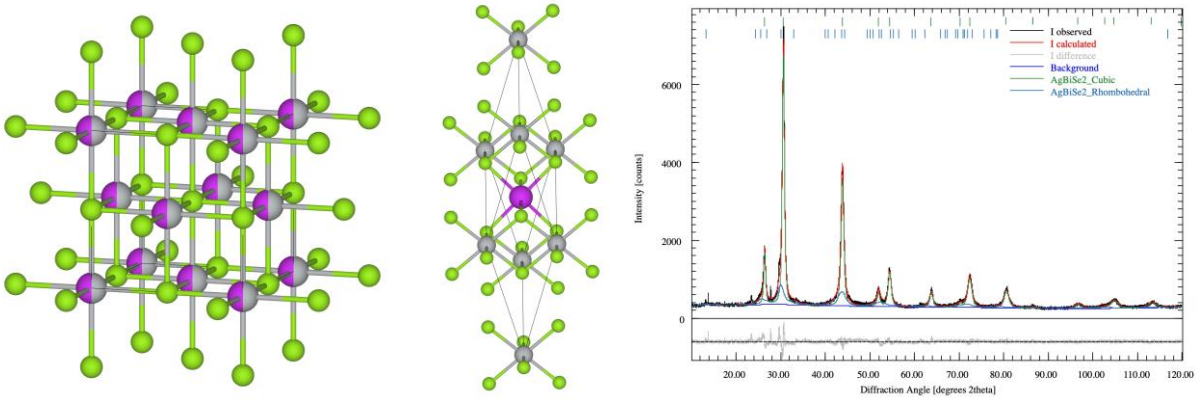
There has been considerable interest in I-V-VI semiconductors (I=Cu,Ag; V=Sb,Bi; VI=S, Se, Te) that are potential candidates for high efficiency photovoltaic cells, due to their NIR band gaps and large absorption coefficients. In DFT calculations performed in the framework of a project aimed at high-throughput *ab-initio* inverse design of materials, $AgBiSe_2$ stood out as particularly promising, given its optical and electronic transport properties. These calculations pointed out for the existence of 3 polytypes, a cubic (*F*m-3m), a rhombohedral (*R*-3m) and a trigonal (*P*3m1) one, the latter appearing to be the one with the lowest energy.

We synthesized nanostructured $AgBiSe_2$ via a green chemistry, solution method, that is advantageous as it can afford small-sized particles with a large surface/volume, thus avoiding the use of the high temperatures needed for the conventional solid-state synthesis. However, the existence of different polytypes of this compound makes the synthetic process challenging when seeking for a single-phase product.

In this work, the Na_2SeSO_3 precursor was prepared by the simple mixing of Se powder and Na_2SO_3 in boiling water under stirring[1]. Subsequently, aqueous $Bi(NO_3)_3$ and $AgNO_3$ suspensions were introduced followed by stirring for 1.5 h to yield the target product. The obtained product was examined by PXRD and SEM/EDS that confirmed the presence of two polytypes (cubic and rhombohedral forms) of $AgBiSe_2$ in approximate 3:1 proportion, both in nanometer size. In the cubic polymorph, the Wyckoff *a* site is randomly occupied by Ag and Bi, in the rhombohedral phase Ag and Bi are fully ordered in two distinct sites.



Scheme or Figure 1: Cubic (left) and rhombohedral (centre) structures of $AgBiSe_2$. Rietveld refinement of PXRD data showing the presence of cubic and rhombohedral forms of nanometer crystalline size.

Acknowledgements

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Polymorphism of trispyrazoylmethanes bearing an oxime moiety prepared by mechanochemistry

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Having in mind the development of genuine sustainable processes, from the synthesis of the catalyst to the catalytic process and the isolation of the reaction products, we explored the use of mechanochemistry[1] for the synthesis of *tris*(pyrazol-1-yl)methanes, also known as *C*-scorpionates, one of the most versatile classes of ligands,[2] their metal complexes and their use as catalysts in the multicomponent copper catalysed alkyne-azide cycloaddition "click" reaction. The solvent-free synthesis of *tris*(pyrazol-1-yl)methanes bearing an oxime group at the C-center, through 1,4-addition of pyrazoles to conjugated α-halogenated nitroso- and azoalkenes,[3] was achieved under ball milling conditions in 30 min in good yields. Recrystallization of the reaction product from different solvents afforded two distinct polymorphs of (*E*)-2,2,2-tris(1*H*-pyrazol-1-yl)acetaldehyde oxime, a monoclinic form that crystallises in the $P2_1/n$ space group, and a triclinic form, crystallising in *P*-1. The structure of the two polymorphs was determined from single-crystal XRD (Figure 1). The molecule has considerable conformational freedom through rotation of the pyrazoyl rings and acetaldehyde oxime moiety around single C—N and C—C bonds, and the two polymorphs feature distinct molecular conformations and hydrogen-bond networks.

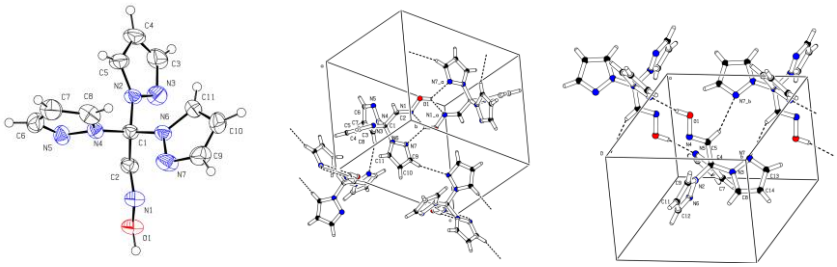

Figure 1: ORTEP plot (at the 50% probability level) of the molecule in the monoclinic polymorph (left) and the hydrogen-bond network in the monoclinic (center) and triclinic polymorphs (right).

Acknowledgements

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3-(Aminomethyl)pyridinium-based organic-inorganic hybrids with halogen-controlled structure

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Often, low-dimensional organic-inorganic hybrids possess photoluminescent (PL) properties with quite high quantum efficiency and tunable band structure.[1] Herein, we present two hybrid compounds, namely, [3AMP] [CdX_4], (3AMP = 3-(Aminomethyl)pyridinium, X = Cl^- (**1**), Br^- (**2**)) based on one-dimensional (1D) chain of $[CdCl_4]^{2-}$ octahedra and zero-dimensional (0D) discrete $[CdBr_4]^{2-}$ tetrahedra, respectively (Figure 1). Both compounds show distinct excitation wavelength-dependent photoluminescence originating from different packing of organic molecules and inorganic units. Our work demonstrates dimensionality modulation by exchange of the halogen atom that might provide effective tunability of PL properties in this low-dimensional hybrids.

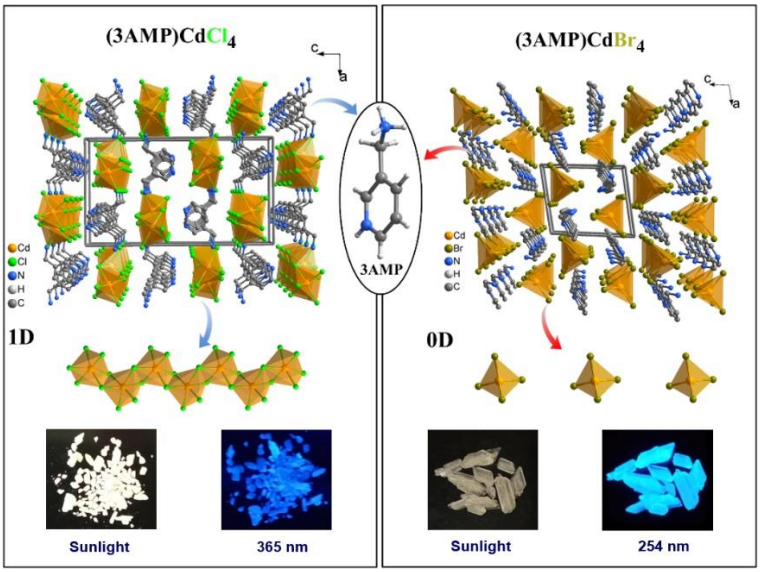

Figure 1: Crystal structures and UV-vis photos of compounds (**1**) and (**2**).

Acknowledgements

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Structural diversity in conducting bilayer salts (CNB-EDT-TTF)$_4$A

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The family of recently described salts based on the electron donor CNB-EDT-TTF (5-cyanobenzene-ethylenedithiotetrathiafulvalene) and different anions A, with general formula (CNB-EDT-TTF)$_4$A, constitutes an unprecedented type of molecular conductors based on a bilayer structure of the donors. The crystal structures of the bilayer conductors (CNB-EDT-TTF)$_4$A with small discrete anions are reviewed and analyzed considering previously reported compounds, with different anions A (corresponding to the linear I_3^-, $AuI_2^-$and I_2Br^- anions, to the tetrahedral ClO_4^-, BF_4^-,and ReO_4^- or to the octahedral PF_6^-, AsF_6^- and SbF_6^- anions). A common structural feature of this family of compounds is the arrangement of the donors head to head, induced by a network of weak C≡N···H-C interactions, which can be described as an effective combination of $R^2_2(10)$ and $R^2_4(10)$ synthons, forming donor bilayers that alternate with the anionic layers. In spite the common donor bilayer arrangement, a rich diversity of structural variations and polymorphs is observed. These structures are related to different types of donor or anion arrangements, which are analyzed. For all anions a β'-type packing pattern of the donor molecules arranged in virtually identical bilayers is observed. However, for (CNB-EDT-TTF)$_4$I$_3$, so far a unique exception in this family of compounds, a distinct κ-type packing of the donors is also obtained as a metastable phase. Another type of polymorphism is observed in β'-(CNB-EDT-TTF)$_4$A, compounds with tetrahedral anions, related with the tilting of the donor molecules (either alternated or uniform) in successive bilayers, leading to monoclinic or triclinic unit cells, with doubling of one cell parameter corresponding to the thickness of one bilayers (~25 Å). Finally, the anions, which often appear with fractional site occupation factors, are expected to be ordered in the anionic plane, with different degrees of correlation between successive anion planes. The implications of these structural features in the electronic properties of these compounds are also discussed.

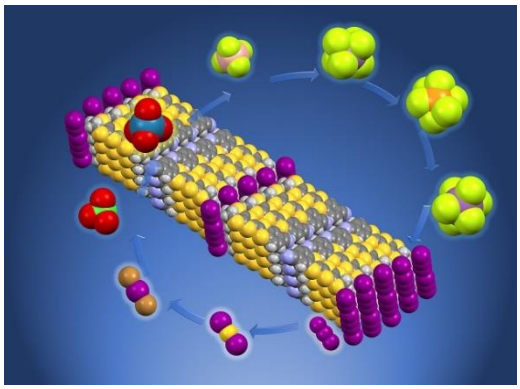

Figure 1: Structural diversity in bilayer salts

Acknowledgements

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The importance of a multi-analytical approach to the study of historical mortars

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The study of historical mortars may bring some challenges in the methodology adopted for their characterization. The limited amounts of sample and the historical importance of the buildings or monuments from which they come may bring limitations to the use of some techniques and tests[1]. In addition, there is often no viability of further collection of new samples, and it is imperative to preserve them. Mortars consist of mixing an inorganic binder with aggregates and water, until a mass is obtained. Once applied, this material reacts with air or water resulting in new mineral phases, especially in relation to the initial raw materials used. The most common binder used in western historical buildings is lime, but gypsum and clays were also used. The studies of historical mortars involve the determination and characterization of the binder and aggregates, being current the use of irreversible processes that require larger amounts of samples, such as acid separation of the binder for the study of the characteristics of the aggregates. Alternatives must be used combining non-destructive techniques. In addition, once applied, these materials may be subjected to a variety of deterioration factors, including their natural aging[2]. The characterization of historical samples can thus be important for determining the conservation state of these materials, as well as assist in decisions related to the actions necessary for their conservation. The methodology proposed in this study includes techniques capable of determining the overall mineralogical and chemical composition of mortars, considering both the binder and the aggregates, such as X-ray fluorescence (XRF) and X-ray diffraction (XRD), as well as the use of optical microscopy, and micro-Raman for the more specific characterization of the aggregates. The preservation of samples makes it possible to use other techniques for more detailed studies of the material, depending on the objective set. For this work, samples of mortars with different functions from the Monastery of Santa Maria de Alcobaça were analysed. The objective of this work is to identify the general characteristics of these materials in their current state of conservation. The results indicate that the use of the specified analytical methodology was sufficient to determine the main characteristics related to mortars, with most of the samples being lime-based mortars and siliceous aggregates, with quartz being the main mineral identified. The samples exposed to environmental conditions showed higher deterioration products, as expected.

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Nitrate in Two Copper(I) Complexes: Ligand or Counterion?

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Insight into the functions of different ligands and how they act within the coordination sphere of a metal complex allows us to understand, and possibly predict their behaviour towards desired applications.[1] Herein we present the synthesis, structural characterisation and DFT study of two novel copper(I) species. These are coordinated by BIAN (bisarylacenaphthenediimine) and triphenylphosphine ligands, with nitrate as a counterion for complex **2** (Figure 1), [Cu(BIAN)(PPh_3)$_2$]NO_3, and as a ligand for complex **1** (Figure 1) [Cu(BIAN)(PPh_3)(ONO_2)], replacing one triphenylphosphine. These complexes were first observed during the preparation of CuAAC (copper catalysed azide alkyne cycloaddition) catalysts and their properties were subsequently explored. Synthetic studies were performed to obtain either species. Both complexes were identified and characterized by single-crystal X-ray diffraction, which will be presented and compared. DFT calculations indicate that **1** can convert into **2** in the presence of free triphenylphosphine via an intermediate.

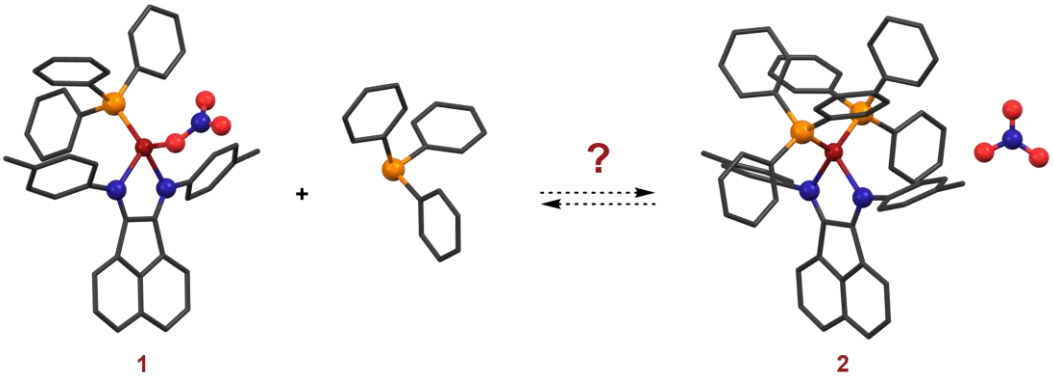

Figure 1: Equilibrium between the copper(I) complexes

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From Cu^{2+} to Li^{+} Fluorescent Chemosensors

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The structural design of a fluorescent ion chemosensor is one of the most important tasks, to achieve the desirable selectivity towards an ion.

With this in mind, a new fluorescent chemosensor for Cu2+ was developed and is going to be presented herein. This sensor is based on a di-(2-picolyl)amine (DPA) as a receptor moiety - responsible to quell metals, and a chalcone backbone - chromophore responsible for the fluorescent signal. Two chemosensors were synthetized, with one or two DPA units, and were fully characterized, including, when possible, single-crystal X-ray diffraction data. Further, UV-Vis and fluorescence titrations were carried out with several ions. Both chemosensors have shown higher selectivity towards Cu2+ through a hypsochromic shift in the absorption spectra. As a consequence, a stronger quenching effect in fluorescence has been noticed. [1]

In a parallel research line, which will be developed during my PhD thesis, we are also focused on the design of new fluorescent chemosensors capable to produce a sensitive response towards lithium presence. Currently, the extraction and processing of lithium have a significant negative impact on the surrounding environment, due to its high toxicity to living wildlife and flora. This significantly highlights the importance of the development of new systems for lithium detection. [2] In this case, the receptor units are cyclic crown ethers with both oxygen and nitrogen atoms that will be coupled to various fluorophores. [3-4]

X-ray crystallography is a powerful and helpful tool that, for both types of systems, allows the confirmation of the molecular structure and ensures the complexation between the chemosensor and the metal center. As a final goal, we aim to establish a relation between the lithium ion coordination and the size of the crown ether required for coordination.

Acknowledgements

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