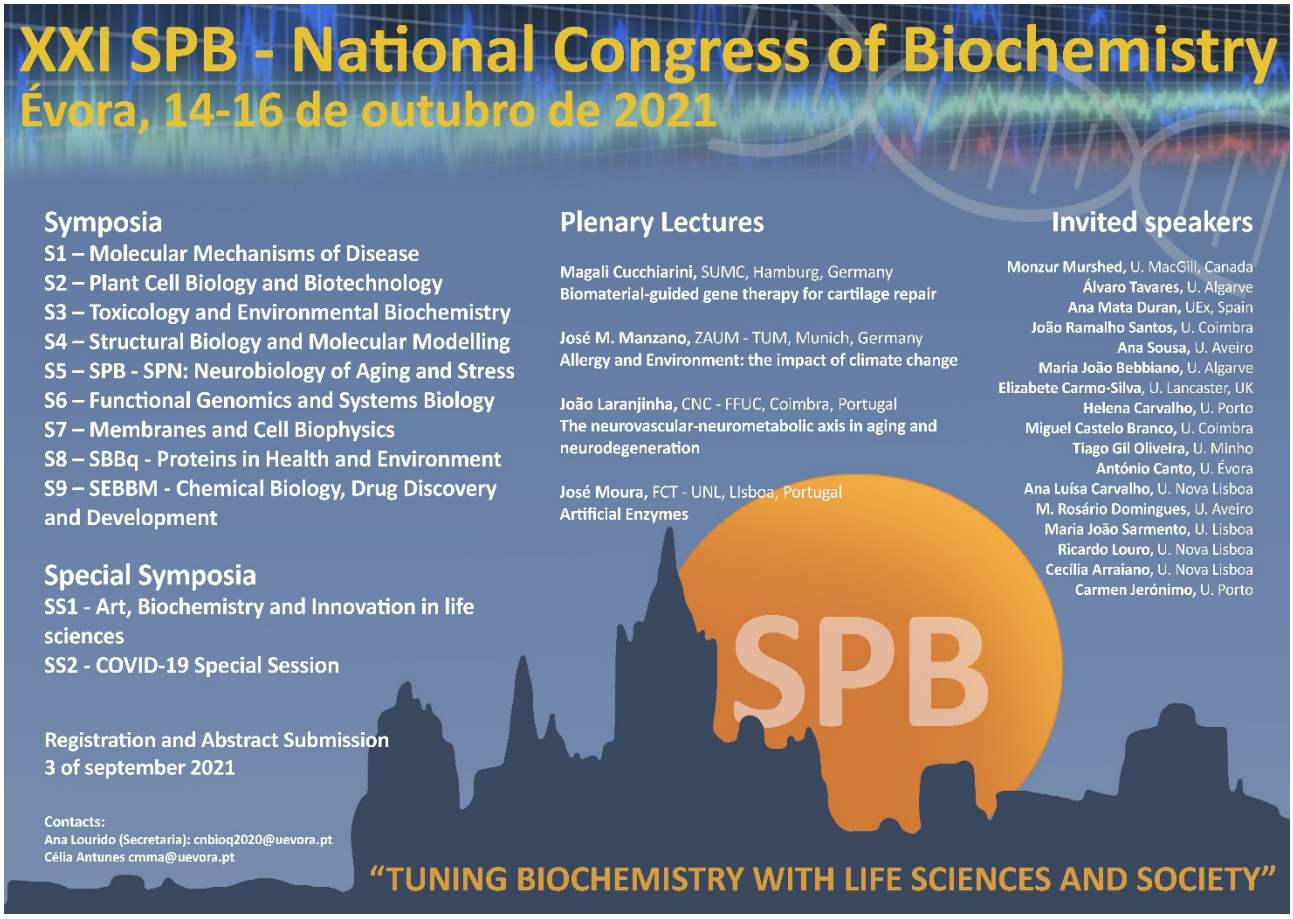
XXI SPB CONGRESS BOOK

14-16 OCTOBER 2021

Évora

Colégio Espírito Santo, University of Évora



Editorial Team

Célia M. Antunes, ESDH/ICT/UÉvora
Ana R. Costa, ESDH/ICT/UÉvora
Patrícia Palma, ICT/IPBeja
Ana Galveias, PhD Student/ICT/UÉvora
Carlos Gastalho, PhD Student/ICT/UÉvora
Mariana Marques, PhD Student/ICT/UÉvora
Leonor Cancela, FMCB/CCMAR/ABC/UAlg
Manuel Aureliano, CCMar/FCT/UAlg

ISBN: 978-972-778-215-4

Published: November 2021

The content of the scientific abstracts is on the responsibility of their respective authors.

Institutional Support and Sponsors

Dear XXI SPB participants,

BioChem, MDPI, will accept the submission of articles from the XXI SPB Congress participants, reviews, conference papers and other high-quality papers. If accepted, BioChem/MDPI offers a 100% discount for papers submitted up to 31/12/2021, 50% discount up to 31/3/2022 and/or other discounts for papers send up to 31/7/2022.

For papers submission, please use the link:

https://www.mdpi.com/journal/biochem/special_issues/Selected_Papers_from_XXI_SPB_National_Congress_Biochemistry_2021

Organizing Committee

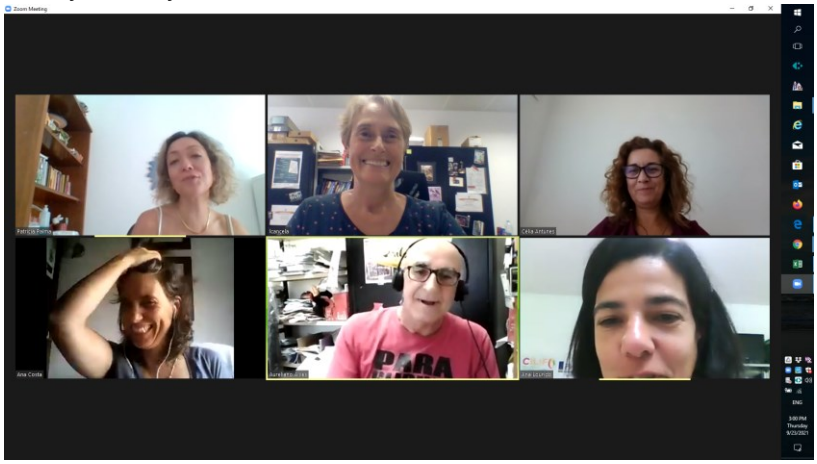
∞

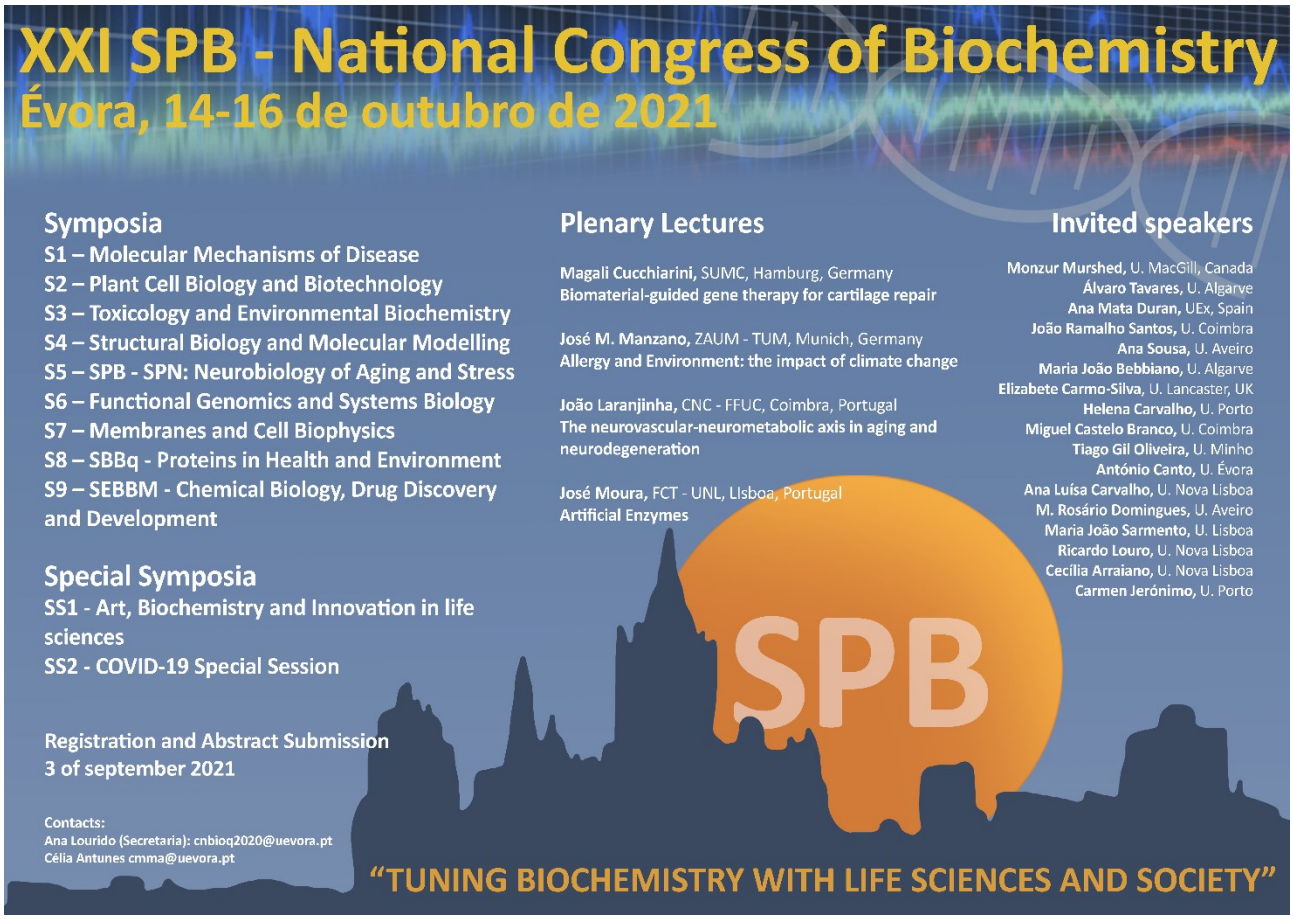
Célia M. Antunes, ESDH/ICT/UÉvora
Ana R. Costa, ESDH/ICT/UÉvora
António Canto, ESDH/CQE/UÉvora
Manuel Aureliano, CCMar/FCT/UAlg
Leonor Cancela, FMCB/CCMAR/ABC/UAlg
Patrícia Palma, ICT/IPBeja

∞

We're going to have to postpone this congress again….

No way, no way! Let's do it in mixed format, from 14-16 Oct! Let's do it!





Scientific Committee

Ana R. Costa, Universidade de Évora
António Canto, Universidade de Évora
Cecília Arraiano, Universidade Nova de Lisboa
Célia M. Antunes, Universidade de Évora
Cláudio Soares, Universidade Nova de Lisboa
Francisco Ambrósio, Universidade de Coimbra
Graça Soveral, Universidade de Lisboa
João Laranjinha, Universidade de Coimbra
Jorge Marques da Silva, Universidade de Lisboa
Leonor Cancela, Universidade do Algarve
Manuel Aureliano, Universidade do Algarve
Manuel Santos, Universidade de Aveiro
Margarida Casal, Universidade do Minho
Miguel Castanho, Universidade de Lisboa
Natércia Teixeira, Universidade do Porto
Nuno Santos, Universidade de Lisboa
Vítor Costa, Universidade do Porto

Acknowledgments

Besides our sponsors the organizing committee would like to thank the following:

LOGO Design
Vera Matos

Secretariat
Ana Lourido

SGE/WEB page
Nelson Madeira

Informatics Support
Joaquim Godinho
Mário Filipe
Ana Filipe
Gonçalo Calhau
Luís Picanço

Accountability
Nuno Malta

Staff
Ana Freire
Ana Luísa Andrade
Ana Silva
António Carrapiço
Carolina Morais
Cristina Mendes
Daniela Sterghite
Diogo Miguel Mataloto
Filipa Santos
Inês Silva
Leoná Correia
Miguel Silvério
Sofia Oliveira

Preface

The University of Évora welcomes YOU at the XXI SPB National Congress of Biochemistry 2020 in **14-16 October 2021** either in person or online!

Under challenging conditions, due to the COVID-19 pandemic, we have managed to organize the National Congress of Biochemistry in a hybrid format, where at least 2/3 of the participants will come to Évora in person. With the pandemic under control and we hope to carry out the Congress both successfully as well as safely.

This is the main meeting point for Portuguese Biochemistry Academy, fostering the discussion and dissemination of high-quality research in Biochemistry, both fundamental and applied, taking place in Portugal. The Scientific Program covers a wide range of issues, from Health and Disease to Environment and Drugs development, where Biochemistry is either fundamental or instrumental in the study of complex and transdisciplinary problems in the society.

The Congress is a moment of Science and Innovation in several Biochemistry domains, sharing experiences and fostering healthy confraternization. Despite the difficult context, over 160 confirmed registrations and >120 abstracts were submitted, involving the whole Portuguese Biochemical community. Moreover, this year for the first time the Congress has gone eco-friendly, with ePosters only where short poster presentations are encouraged.

We hope the congress meets your expectations!

The Organizing Committee, on behalf of the Portuguese Biochemical Society, looks forward meeting you, at Colégio do Espírito Santo, University of Évora!

Célia Antunes, Chair

(Department of Health and Medical Sciences, School of Health and Human Development & ICT) (UEvora)

Message to SPB Members CNB2021

Dear Members of the Portuguese Biochemical Society (SPB), dear Colleagues and Students,

On behalf of the Directive Board of SPB, it is my pleasure to welcome you to the XXI National Congress of Biochemistry.

I would like to address a special thanks to Dr. Célia Antunes, Dr. Ana Costa and all the Organizing Committee, for accepting the challenge of organizing the Congress of SPB despite the difficult times imposed by the COVID-19 pandemic. Although several scientific meetings are being successfully held on virtual mode, the advantages of face-to-face communication for presenting research work and networking are unquestionable. It is our hope that the hybrid mode adopted for the SPB 2021 Congress will attract a larger number of students and senior scientists, helping potentiate networking and generate new synergies and future collaborations, to foster the scientific careers of young researchers.

I would like to highlight the excellence of the Scientific Program with the motto “Tuning Biochemistry with Life Sciences and Society” covering a broad range of topics, from molecular mechanisms of disease to environmental toxicology and society challenges, demonstrating the high standards of Portuguese Biochemistry.
Thanks to the contribution of outstanding scientists as Plenary and Symposium speakers and to the close collaboration with our partner Societies Brazilian Society of Biochemistry and Molecular Biology (SBBq), Spanish Society of Biochemistry and Molecular Biology (SEBBM), and Portuguese Society for Neuroscience (SPN) that were crucial for the co-organization of three ssymposia, this Congress presents a great opportunity to present and discuss scientific work in an international environment where all participants, researchers, students and professionals, can exchange ideas and establish collaborations.

I hope you will enjoy the scientific and friendly environment provided by the University of Évora.
Thank you all for attending the National Congress of SPB!

Graça Soveral
The President, Portuguese Biochemical Society

Program

14 October 2021 (Thursday)

13:00h	**Registration**	
14:30h	**Opening Ceremony**	
15:00h	**PLENARY LECTURE 1** **Magali Cucchiarini,** Saarland University Medical Center (Germany) "*Biomaterial-Guided Gene Therapy for Cartilage Repair*" ***Chair*: Leonor Cancela (UAlg)**	
16:00h	**Coffee break**	
-	**Auditorium ES**	**Amphitheatre ES**
16:30h	**S1 – Molecular Mechanisms of Disease** *Chairs*: Leonor Cancela (FMCB-UAlg); Magali Cucchiarini (Saarland University); Manuel Aureliano (FCT-UAlg)	**S2 - Plant Cell Biology and Biotechnology** *Chairs*: Jorge M. Silva (FCUL); Teresa Lino-Neto (UMinho)
	Invited lectures: IL1, Monzur Murshed (MacGill Univ., Canada) *"Understanding the pathobiology of Keutel Syndrome"* IL2, Álvaro Tavares (UAlg) *"Control of cell proliferation"*	**Invited lectures:** IL3, Elizabete Carmo-Silva (Lancaster Univ., UK) *"Rubisco activase isoform diversity and crop adaptation to dynamic environments"* IL4, Helena Carvalho (UP) *"Unravelling the function of glutamine synthetase of the prokaryotic type (GSI-like) in nitrogen signalling in Medicago truncatula"*
17:30h	**Oral Communications OS1a** (OC1-OC6)	**Oral Communications OS2** (OC7-OC11)
18:30h	**Flash communications PS1a** (FC1-FC8) *Chairs*: Manuel Santos (UAv); Álvaro Tavares (UAlg)	**Flash communications PS2** (FC9-FC21) *Chairs*: Jorge M. Silva (UL); Helena Carvalho (UP)
19:30h	**Welcome reception**	

15 October 2021 (Friday, morning)

<table>
<tr><td>09:00h</td><td colspan="2">PLENARY LECTURE 2
José Maria Manzano, ZAUM, TUM (Germany)

“Environmental allergens, climate change and health impact”

Chair: Célia M Antunes (UÉv)</td></tr>
<tr><td>10:00h</td><td colspan="2">Coffee Break</td></tr>
<tr><td>-</td><td>Auditorium ES</td><td>Amphitheatre ES</td></tr>
<tr><td>10:30h</td><td>S3 - Toxicology and Environmental Biochemistry

Chairs: Ana R. Costa (UÉv); José Maria Manzano (TUM, Germany)</td><td>S4 - Structural Biology and Molecular Modelling / S7 – Membranes and Cell Biophysics

Chairs: Cláudio Soares (ITQB-UNL); Graça Soveral (UL); Nuno Santos (FMUL)</td></tr>
<tr><td></td><td>Invited lectures:

IL5, Ana Catarina Sousa (UÉv)

“Hormones messed up - a tale of endocrine disruptors in the XXI century”

IL6, Maria João Bebbiano (UAlg)

“Are micro and nanoplastics toxic?”</td><td>Invited lectures:

IL7, Ana Luísa Carvalho (UNL)

“A molecular view on the carbohydrate preferences of highly efficient cellulolytic bacteria”

IL8, M. Rosário Domingues (UAv)

The use of lipidomics to study membrane phospholipids”</td></tr>
<tr><td>11:30h</td><td rowspan="2">Oral Communications OS3
(OC12-OC16)</td><td>IL9, Maria João Sarmento (CAS, Czeck Republic)

“The impact of the glycan headgroup on the nanoscopic segregation of gangliosides”</td></tr>
<tr><td>12:00h</td><td>Oral Communications OS4/S7
(0C17-OC19)</td></tr>
<tr><td>12:30h</td><td>Flash communications PS3
(FC22-FC30)

Chairs: Maria João Bebbiano (UAlg); Patrícia Palma (IPBeja)</td><td>Flash communications PS4/S7
(FC31-FC40)

Chairs: Cláudio Soares (ITQB NOVA); Graça Soveral (FFUL); Nuno Santos (FMUL)</td></tr>
<tr><td>13:00h</td><td colspan="2">Lunch</td></tr>
</table>

15 October 2021 (Friday, afternoon)

<table>
<tr><td>14:00h</td><td colspan="2">PLENARY LECTURE 3
João Laranjinha, CNC, FFUC

“The neurovascular-neuroenergetic axis in aging and neurodegeneration: the key role of nitric oxide”

Chairs: Francisco Ambrósio (UC); Vítor Costa (UP)</td></tr>
<tr><td rowspan="2">15:00h</td><td>S5 - SPB-SPN: Neurobiology of aging and stress

Chairs: Ana Cristina Rego (UC); João Laranjinha (UC)</td><td>S6 - Functional Genomics and Systems Biology

Chairs: Cecília Arraiano (UNL); Sandra Viegas (ITQB-UNL)</td></tr>
<tr><td>Invited lectures:

IL10, Tiago Oliveira (UMinho)

“The impact of chronic stress on the brain lipidome – implications for mood disorders and Alzheimer’s disease”

IL11, Miguel Castelo Branco (UC)

“In vivo functional and molecular imaging approaches to study ageing and neurodegenerative disorders”</td><td>Invited lectures:

IL12, Cecília Arraiano (UNL)

“The Expanding Universe of RNAs”

IL13, Carmen Jerónimo (UP)

“DNA and RNA methylation in urological cancer: from biology to clinical biomarkers”</td></tr>
<tr><td>16:00h</td><td>Oral Communications OS1b/S5
(OC20-OC24)</td><td>SS1 – Art, Biochemistry, and Innovation in Life Sciences

Chairs: Leonor Cancela (UAlg), Manuel Aureliano (UAlg), Célia Antunes (UÉv)

i) Biochemistry goes to School: inovative projects for Biochemistry dissemination
▪ José Bragança (UAlg)
▪ Márcio Simão (UAlg)

ii) Education in Action: Biochemistry olimpiads and IDays
▪ Renato Simões, Margarida Quadros (UC)
▪ Carlos Godinho (UÉv)

iii) Biochemisty and Society: Biochemistry in Art and Heritage
▪ Cátia Lavrador et al. (UÉv)
▪ Teresa Fernandes & Célia Lopes (UÉv)</td></tr>
<tr><td>17:00h</td><td colspan="2">Coffee Break</td></tr>
<tr><td>17:30h
18:30h</td><td>Flash communications PS1b/S5
(FC41-FC52)

Chairs: Tiago Oliveira (UMin), João Laranjinha (UC)</td><td>Flash communications PS6/S8/S9
(FC53-FC66)

Chairs: Ricardo Louro (ITQB-UNL), João Ramalho (UC)</td></tr>
<tr><td>18:30h</td><td colspan="2">Guide Trip to the Historical Centre</td></tr>
<tr><td>20:00h</td><td colspan="2">Congress Dinner</td></tr>
</table>

16 October 2021 (Saturday)

	Auditorium ES	Amphitheatre ES
09:00h	**S8 - SBBq - Proteins in Health and Environment** *Chairs*: Miguel Castanho (UL); José Moura (UNL)	**S9 - SEBBM – Chemical Biology, drug discovery and development** *Chairs*: Carlos Gutierrez-Merino (UEx, Spain); Manuel Aureliano (UAlg)
	Invited lectures: IL14, Ricardo Louro (ITQB-UNL) *"Metalloproteins fighting infection and climate change: Iron-cold iron, is master"* IL15, Eduardo Sousa (Federal University of Ceará, Brasil) *"Oxygen heme-based sensors in nature and medicine"*	**Invited lectures:** IL16, Ana Mata Duran (UEx , Spain) *"The emerging role of methylene blue in the interplay between PMCA and biomarkers of Alzheimer's disease."* IL17, João Ramalho Santos (UC) *"I want a new drug: Searching for novel modulators of sperm function."*
10:00h	**Oral Communications S8** (OC25-OC30)	**Oral Communications S9** (OC31-OC34)
11:00h	**Coffee Break**	
11:30h	**PLENARY LECTURE 4** **José Moura (FCT-UNL)** *"Bioinorganic Chemistry and Design of Artificial enzymes"* ***Chair:* Manuel Aureliano (UAlg)**	
12:30h	*Best Oral and Poster Communication Award Ceremony*	
13:00h	**Lunch**	
14:45h	**SS2 – COVID Special Session (PT)** *Chairs*: Victor Ramos (Diretor da ESDH, UÉv); Graça Soveral (FFUL, Presidente da SPB)	
	▪ Carlos Pinto Sá - Presidente da Câmara Municipal de Évora – Respostas e desafios sociais do município; ▪ Ana Costa Freitas, Reitora da U. Évora - Respostas e desafios do Ensino Superior; ▪ Ricardo Mexia, ANMSP – Desafios ao nível da Saúde Pública: aprendizagens e estratégias de prevenção; ▪ Isabel Pita, HESE – Desafios para a gestão de um Hospital Público/SNS em Pandemia; ▪ Miguel Castanho, FMUL – Desafios de natureza molecular na compreensão da COVID-19: o papel da bioquímica presente e futuro;	
17:00h	**Closing Ceremony**	

Index

Symposia

Symposium 1 – Molecular Mechanisms of Disease

Proper functioning of cells requires robust control mechanisms. These ultimately rely on complex signalling pathways that mark the crossroads between physiology and pathology. Understanding the events at the molecular level will enable the discovery of new targets and development of innovative strategies towards personalised molecular medicine in non-communicable diseases. During this session, new findings in above-mentioned control mechanisms and systems will be explored and discussed.

Symposium 2 –Plant Biology and Biotechnology

With an expected world population of almost 10 billion by 2050, and increasingly harsh conditions to agricultural production due to climate change, significant advances in plant sciences are needed to cope with global food security. A more comprehensive understanding of the interactions between plant, environmental conditions and cultural practices is needed. To achieve this goal, a better integration of knowledge at different levels, from molecular biology to whole-plant physiology, is required.

Within this session, we will get aware on the diversity of current approaches to in plant sciences, from new breeding techniques to high-throughput plant phenotyping or plant ecological networks.

Symposium 3 - Toxicology and Environmental Biochemistry

Toxin-poison and other bioactive substances, whether chemically synthesized or produced by living organisms, such as bacterial toxins, mycotoxins, phycotoxins or zootoxins, allergens or environmental pollutants may trigger several responses within the biochemical machinery of the organisms, unleashing diverse AOPs. The exposome is a determinant key for many undesired outcomes.

The session will cover several components of the exposome, their target biomolecules, cell responses and biomarkers, the development of new detection methods for toxins, allergens, and pollutants as well as the development of mitigation strategies.

Symposium 4 - Structural Biology and Molecular Modelling

Cellular function is supported by finely orchestrated biomolecular interactions. Macromolecular assemblies coordinate complex biological functions such as transcription, translation, cytoskeleton organization, signalling cascades and secretion pathways. To fully grasp the underlying complexity and dynamics of these molecular machines the combination of integrative Structural Biology and Molecular Modelling approaches is crucial. During this session, experimental and theoretical structural biology studies will be presented revealing the molecular mechanisms of various biomolecules with unprecedented atomic detail.

Symposium 5 - SPB-SPN: Neurobiology of aging and stress

Molecular and physiological changes associated with neurodegenerative diseases are inexorably linked with aging. Age-related gradual decline of organismal homeostasis, which includes changes in ER and oxidative stress, mitochondrial dysfunction, DNA damage, intracellular transport, protein synthesis and turnover, etc., have all been linked with formation of pathognomonic protein aggregates. Thus, advances in our understanding of the molecular basis of neurodegeneration and healthy aging present an imperative and exciting opportunity to identify novel druggable targets and develop more effective interventions.

Symposium 6 - Functional Genomics and Systems Biology

The structure, regulation and integrity of genome are essential for cell division, organims´ development and adaptation to environmental stimuli. With Biology entering the digital era, we are immersed in a wealth of information originating in high-throughput experiments. These data enable us for the first time to study life at the level unimaginable before and understand the entire living systems from molecules to cells, from organisms and communities to ecosystems. This session will outline the great diversity in the epigenomics field, DNA regulation and remodeling, DNA and RNA methylation, new ways of post-translational modifications, by presenting data from diverse model systems clinical research and new insights in clinical research.

Symposium 7 – Membranes and Cell Biophysics

Cell membranes are essential to cell function and are crucial for life. They establish an external barrier separating the inner from the outer media and define intracellular compartments (organelles) essential for metabolic processes and molecular trafficking. The coordinated regulation of membrane components (lipids and proteins) has impact in membrane selective permeability and is a feature providing flexibility and robustness to a variety of physiological processes, including cell signalling. Disorders of membrane lipids, dysfunction of membrane proteins and disruption of cell compartmentalization have serious consequences for living cells and organ performance and are related with several diseases. Several of these issues, from the structural to the functional level, will be addressed in this session.

Symposium 8 - SBBq - Proteins in Health and Environment

Virtually all the important cellular processes such as cell division, cell motility, organelle remodelling, and intracellular trafficking depend on the precise localization of proteins and the dynamics of their interactions. Protein folding and trafficking pathways in prokaryotes and eukaryotes are among the most fundamental processes in all of biology. Understanding the mechanisms of protein folding and misfolding regulation and protein trafficking is crucial to understand the role of proteostasis in physiological and pathological conditions, ranging from metabolic imbalance and aging, neurodegeneration, infections, immune disorders, tumorigenesis, and cancer therapy.
This session will cover the topics of protein localization, structure, and dynamics, by different approaches from biophysics to cell biology, including their impact on human disease focusing on their impact on human diseases and contribution to diminish the ecological footprint of human activities.

Symposium 9 - SEBBM - Chemical Biology, Drug Discovery, and Development

Medicinal chemistry is a highly multidisciplinary and rapidly developing area that deals with the research on biologically active compounds and their interactions with biological targets to understand their mode of action at the molecular level, and how they can be included in clinically relevant formulations. This session will provide insights into recent advances on different techniques for design and preparation of new drug leads in antiviral, antimicrobial, and anticancer chemotherapy, as well as new drug candidates for contraception, neurodegenerative and inflammatory diseases. Emphasis will be focused on identification of multitarget-directed small molecules as innovative therapeutic tools, and best administration routes. Mechanism of action and structure–activity relationship studies, including elucidation of target enzyme/protein-drug interaction and metabolic pathways will be discussed.

Special Session 1

In the last years Science and Art have been merging to create innovative views into the world. New paradigms where science, art and intervention strategies interlace fostering and innovation projects in:

1. Biochemistry goes to School: innovative projects for Biochemistry dissemination.
2. Education in Action: Biochemistry Olympiads and iDays;
3. Biochemistry and Society: Biochemistry in Art and Heritage.

Special Session 2

The COVID-19 pandemic has challenged the community globally, profoundly defying our way of living.
The objective of this session is to present the characteristics of this pandemic and discuss the challenges facing government entities, institutions, the scientific community, and the public. It also intends to contribute to the construction of new perspectives, enhancing interdisciplinary approaches in the context of interventions in Public Health.

PLENARY LECTURES

(PL1-PL4)

Magali Cucchiarini *Center of Experimental Orthopaedics, Saarland University and Saarland University Medical Center, Kirrbergerstr*	Biomaterial-Guided Gene Therapy for Cartilage Repair
José Maria Maya Manzano *Center for Allergy and Environment (ZAUM), Technical University and Helmholtz Center Munich, Germany.*	Environmental allergens, climate change and health impact
João Laranjinha *Faculty of Pharmacy and Center for Neurosciences and Cell Biology, University of Coimbra, Portugal*	The neurovascular-neuroenergetic axis in aging and neurodegeneration: the key role of nitric oxide
José J. G. Moura *LAQV, REQUIMTE, Nova School of Science and Technology (FCT NOVA), Caparica, Portugal*	Bioinorganic Chemistry and Design of Artificial enzymes

Chairs: Leonor Cancela (UAlg); Célia M. Antunes (UÉv); Franscisco Ambrósio (UC); Vitor Costa (UP); Manuel Aureliano (UAlg)

PL1 - Biomaterial-Guided Gene Therapy for Cartilage Repair

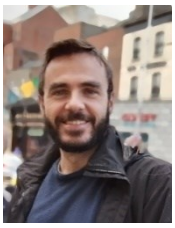
Cucchiarini M.

Center of Experimental Orthopaedics, Saarland University and Saarland University Medical Center, Kirrbergerstr. Bldg 37, D-66421 Homburg/Saar, Germany

Email: mmcucchiarini@hotmail.com

Background. Articular cartilage defects have a limited self-ability to fully heal and may evolve to irreversible osteoarthritis if left untreated. While a number of options are available in the clinics to treat such defects in patients, none of them satisfactorily and definitely address the problem of incurable cartilage damage. Biomaterial-guided cartilage gene therapy is an emerging, highly promising field of research to durably and safely manage cartilage degeneration via the spatial and temporal delivery of reparative gene sequences using vectors transferred to sites of cartilage injury via biocompatible materials including hydrogels, solid scaffolds, and hybrid compounds. This work will present the most up-to-date findings in the field of scaffold-assisted cartilage gene therapy with a focus on recent findings in clinically relevant animal models of cartilage injury *in vivo* as a future off-the-shelf, non-invasive tool to treat affected individuals in clinical protocols.

PL2 - Environmental allergens, climate change and health impact



José María Maya Manzano

Center for Allergy and Environment (ZAUM), Technical University and Helmholtz Center Munich, Germany.

Email address: jose.manzano@tum.de

Background. Environmental allergens outdoors (as those contained into the pollen grains from anemophilous plants or spores from fungi) and indoors (originated from house dust mites and pets) are studied by Aerobiology. This multidisciplinary science comprehends a vast (and often unknown) number of applications benefiting medical doctors, allergy patients, urban planners and stakeholders. Moreover, it aims to have a deeper understanding of how environmental processes are controlling or limiting the presence of allergens and specifically for pollen, also their Pollen Allergen Potency (*PAP*). In addition, we should consider that plants producing allergenic pollen also change their distribution along the time both locally and regionally, which represents a major concern for all the scientists involved in the allergens´ study.

Methods. When aeroallergens are studied, their sampling usually involve cascade impactors, varying the model depending on the target environment. Other recent techniques, as air filtration, also work to remove allergens and help to mitigate allergy symptoms in those scenarios that hardly can be improved in other ways (exposure to allergens in indoor environments). Also, the creation of risk maps showing potential exposure to allergens is another tool of interest. Finally, by using diverse modelling techniques integrating future possible scenarios, it has been possible to predict how the exposure to *Betula* pollen grains (and thus their allergens) in the future of Bavaria (Germany) will be.

Results. Here are shown the results of some studies regarding different aspects of allergens and their sources, their distribution along the pollen season, its distribution for each size fraction, how it varies for each place and some drivers that control their presence in the environment. Moreover, and in higher time scale, in Bavaria the climate change is expected to increase the birch pollen load in short-term, but, due to the future reduction in birch trees also provoked by their effects, the airborne birch pollen concentrations will finally decrease at lower altitudes. On the contrary, areas with higher altitude will have an increase in the surface occupied by birch trees and subsequent it will lead in higher airborne birch pollen concentrations in the future. Regarding indoor allergens, portable air filtration devices can be effective in reducing exposure to airborne indoor allergens and particle matter, which might be beneficial to breathing troubled patients.

Conclusions. The findings presented here can be used by allergists to optimize allergy treatments depending on the PAP, the risk of exposure or future changes in species distribution provoked by climate change effects. Also informs about the presence of possible solutions to deal with airborne indoor allergens. To understand the complexity for all factors affecting the allergen concentrations and how it varies with time and space (and their implications) are key in order to help allergy patients to prevent unnecessary exposure and for scientist to get ready to face future and unexpected challenges.

PL3 - The neurovascular-neuroenergetic axis in aging and neurodegeneration: the key role of nitric oxide

João Laranjinha[1,2], Cátia Lourenço[2], Ana Ledo[1,2], Carla Nunes[2], Ricardo Ferreira[2], Cândida Dias[2], João Gonçalves[2] and Rui Barbosa[1,2]

[1]Faculty of Pharmacy and [2]Center for Neurosciences and Cell Biology, University of Coimbra, Portugal

Email address: laranjin@ci.uc.pt

Background

Owing to its high energy needs, the brain is endowed with fine mechanisms for a precise spatial and temporal control of cerebral blood flow (CBF) according to neural activity, the neurovascular coupling (NVC) also referred to as functional hyperemia. Failure at any part of the NVC pathway could cause disrupted CBF resulting in catastrophic depletion of oxygenation and energy supply.

The search for modulators of NVC has been a challenge over the years. By developing innovative tools for in vivo assessment of NVC we have described neuronal nitric oxide (NO), along the NMDAreceptor-nNOS-NO pathway, as direct mediator of the communication between neurons and local microvessels (Lourenço et al 2014). Paradoxically, local increases of NO upon neuronal activation sets the stage for its competition with oxygen for reversible binding to mitochondrial cytochrome c oxidase. Thus, NO is central to the regulation of the neurovascular-neuroenergetic coupling axis in the brain (Lourenço et al 2017b). The functionality of NVC is key for cognitive performance and becomes impaired during aging and age-associated neurodegeneration, notably Alzheimer´s disease (Lourenço et al. 2017a). In addition, we have come to conjecture that the redox and functional interplay of nitric oxide with ascorbate and nitrite would modulate the functionality of glutamatergic synapses in terms of NVC. As such, this might be an operative mechanism in the brain maintaining and enhancing cognitive performance in aged individuals.

Methods

We have used a multimodal approach, comprising microarrays for stereotaxic insertion in the brain of living rodents, consisting of microinjection pipettes, laser Doppler blood flow probes and selective microelectrodes, behavior and biochemical approaches to probe the dynamics and functional impact of O_2, glucose, NO, ascorbate and CBF in vivo in terms of NVC in animal models of aging and AD disease.

Results and conclusions

Data in vivo in rodents support that (1) neuronal-derived NO acts as a direct mediator of neurovascular coupling, (2) neurovascular coupling is impaired in Alzheimer´s and aging due to vascular dysfunction, 3) the redox interaction of nitrite/ascorbate/NO functionally coupled to neuronal activation supports neurovascular coupling, 4) rescue from impaired neurovascular coupling results in enhancement of cognitive performance.

Overall, cognitive impairment might be averted by sustaining the bioavailability of NO, the critical messenger coupling neuronal activity with CBF increases.

References

Lourenço et al (2014) Neurovascular coupling in hippocampus is mediated via diffusion by neuronal-derived nitric oxide. *Free Radical Biology and Medicine 73, 421-429.*

Lourenço et al. (2017a) Neurovascular uncoupling in the triple transgenic model of Alzheimer´s disease: impaired cerebral blood flow response to neuronal-derived nitric oxide signaling. *Experimental Neurology* 291, 36-43.

Lourenço et al (2017b) Neurovascular-neuroenergetic coupling axis in the brain: master regulation by nitric oxide and consequences in aging and neurodegeneration. *Free Radical Biology and Medicine* 108, 668-682.

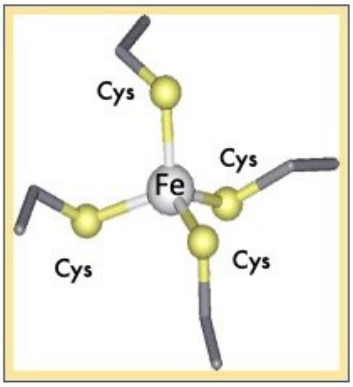
PL4 - Bioinorganic Chemistry and Design of Artificial enzymes

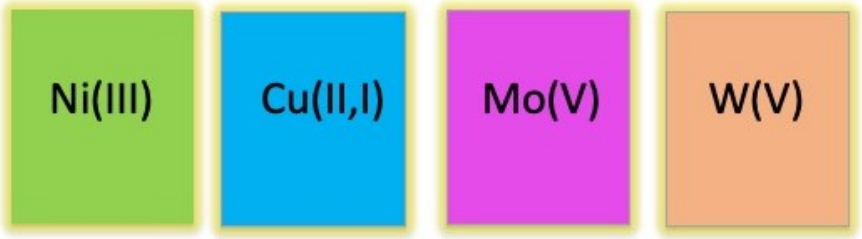
José J. G. Moura

LAQV, REQUIMTE, Nova School of Science and Technology (FCT NOVA), Caparica, Portugal
Email: jose.moura@fct.unl.pt

Enzymes are complex molecules that carried on chemical transformations with amazing selectivity and at high rates. Metalloproteins and metal-containing enzymes are well known to be essential to life. The elucidation of structural and functional aspects of metal sites in enzymes has been a goal of model studies bringing together Inorganic Chemistry and Synthetic Biochemistry related to the developing area of artificial enzymes. In particular, synthetic peptides and small proteins involving rich sulfur coordination sites have been extensively promising and used, such as Rubredoxins (Rds) and analogues. The four-Cysteine metal coordination motif, available in Rds, has the possibility of coordinating a wide variety of transition metals (Fe, Ni, Cu, Mo and W), with particular interest in modelling complex metalloproteins most relevant in energy, environment, agriculture and health topics.

Acknowledgments
Biplab K Maiti, Pedro Bragança, Cintia Carreira, Maddalena Elia, Célia Silveira, Smilja Todorovic, Marta S. P. Carepo, Sofia R. Pauleta, Luisa B. Maia, and Isabel Moura are aknowledged for many experimental contributions. JJGM received financial support from PT national funds (FCT/MCTES) through projects PTDC/BTA-BTA/0935/2020, UIDB/50006/2020 and UIDP/50006/2020.





SYMPOSIUM 1 – MOLECULAR MECHANISMS OF DISEASE

Proper functioning of cells requires robust control mechanisms. These ultimately rely on complex signalling pathways that mark the crossroads between physiology and pathology. Understanding the events at the molecular level will enable the discovery of new targets and development of innovative strategies towards personalised molecular medicine in non- communicable diseases. During this session, new findings in above-mentioned control mechanisms and systems will be explored and discussed.

IL1
Monzur Murshed (MacGill University)

IL2
Álvaro Tavares (UAlg)

INVITED LECTURES S1

(IL1-IL2)

Monzur Murshed *Department of Medicine and Faculty of Dentistry, McGill University, Montreal, Canada*	Understanding the pathobiology of Keutel Syndrome
Alvaro Tavares *Department of Biomedical Sciences and Medicine, University of Algarve, Portugal*	Control of cell proliferation

Chairs: Leonor Cancela (FMCB_UAlg); Magali Cucchiarini (Saarland University); Manuel Aureliano (FCT-UAlg)

IL1 - Understanding the pathobiology of Keutel Syndrome

Monzur Murshed, PhD

Professor, Department of Medicine and Faculty of Dentistry, McGill University, Montreal, Canada
Researcher, Shriners Hospital for Children, Montreal, Canada
Email address: monzur.murshed@mcgill.ca

MGP is a small extracellular matrix protein which acts as a strong inhibitor of abnormal 'soft' tissue calcification, particularly in the cartilaginous and vascular tissues. MGP deficiency in humans leads to a rare autosomal recessive disorder known as Keutel Syndrome (KS). These patients show mineralization of all cartilaginous tissues, including the nasal septum, growth plates and the trachea, leading to abnormal skeletal development and respiratory ailments. In addition, vascular calcification has been reported in a small number of KS patients. Our group has used genetically modified mouse models to understand the pathobiology of KS. We have shown that midface hypoplasia in MGP-deficient mice is associated with nasal septum calcification, apoptosis of the septal chondrocytes and premature closure of the spheno-occipital synchondrosis. In a separate study, we demonstrated that the severity of vascular calcification in MGP-deficient mice depends on the amount of elastin present in the arterial walls. We further showed that MGP's two post translational modifications – phosphorylation of three conserved serine residues, and carboxylation of 4 conserved glutamic acid residues contribute to MGP's anti-mineralization function in vivo.

IL2 - Control of cell proliferation

Santos I.B.[1,2], Garrido-Maraver, J.[1,2] and Tavares, AA[1,2]

[1] Department of Biomedical Sciences and Medicine, University of Algarve, Portugal. [2] Centre for Biomedical Research (CBMR), University of Algarve, Portugal
Email address: aatavares@ualg.pt

The regulation of growth through signaling pathways plays a critical role in maintaining tissue homeostasis through the regulation of key cellular processes like cell proliferation and cell death. Signaling pathways comprise of cascade of regulatory proteins that respond to stimulators like growth factors, and influence changes in gene expression that control differentiation, cell migration, cell–cell interaction, immunity, polarity, and metabolism.

Disruption of signaling pathways causes an imbalance in the regulation of such mechanisms and leads to diseases such as neuro/muscular degeneration, cancer, diabetes, etc. The Hippo Pathway is a prime example of an important growth regulatory pathway that coordinately controls cell proliferation and survival to regulate organ size.

Hippo pathway was initially identified through genetic screens for genes regulating organ size in fruitflies. Hippo pathway is comprised of genes that act as tumor suppressor genes like hippo (hpo), warts (wts) and Mob1, and oncogenes like yorkie (yki). YAP and TAZ are two related mammalian homologs of Drosophila Yki that act as effectors of the Hippo pathway. Hippo signaling deficiency can cause YAP- or TAZ-dependent oncogene addiction for cancer cells. YAP and TAZ are often activated in human malignant cancers. These transcriptional regulators may initiate tumorigenic changes in solid tumors by inducing cancer stem cells and proliferation, culminating in metastasis and chemo-resistance. Given the complex mechanisms (e.g., of the cancer microenvironment, and the extrinsic and intrinsic cues) that overpower YAP/TAZ inhibition, the molecular roles of the Hippo pathway in tumor growth and progression remain poorly defined.

I will sumarize our recent findings from studies in whole animal model organism (Drosophila) and human cultured cells on the role of Mob-like proteins regarding their interaction with the Hippo signaling pathway and their connection to tumor formation and progression.

ORAL COMMUNICATIONS S1a

OC1	Maria Brito	Breast cancer brain metastases: molecular mechanisms, cellular interplay and peripheral biomarkers
OC2	Manuel Aureliano	Comparison of SERCA and PMCA inhibition potential of polyoxotungstates
OC3	Rui Dinis	Identification of germline mutations in patients with breast and difuse gastric cancer in Alentejo
OC4	Inês V. da Silva	Peroxiporins and pancreatic cancer: new roles for aquaporin-3 and aquaporin-5 in tumor biology
OC5	Ana Quendera	The RNA-binding protein PNPase is a novel regulator of biofilm formation and virulence in Listeria monocytogenes
OC6	Mafalda Migueis	The role of sacsin in the pathological aggregation of intermediate filaments

Chairs: Leonor Cancela (FMCB_UAlg); Magali Cucchiarini (Saarland University); Manuel Aureliano (FCT-UAlg)

OC1 - Breast cancer brain metastases: molecular mechanisms, cellular interplay and peripheral biomarkers

Figueira I[1,2], Godinho-Pereira J[1,3], Custódio-Santos T[1], Galego S[1], Maia J[4,5], Haskó J[6], Molnár K [6], Malhó R[7], Costa-Silva B[4], Wilhelm I[6,8], Krizbai IA[6,8] and Brito MA[1,3]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal; [2]Farm-ID – Associação da Faculdade de Farmácia para a Investigação e Desenvolvimento, Lisbon, Portugal; [3]Faculty of Pharmacy, Universidade de Lisboa, Portugal; [4]Champalimaud Centre for the Unknown, Lisbon, Portugal; [5]Graduate Program in Areas of Basic and Applied Biology, Universidade do Porto, Portugal; [6]Institute of Biophysics, Biological Research Centre, Eötvös Loránd Research Network (ELKH), Szeged, Hungary; [7]BioISI, BioSystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Portugal; [8]Institute of Life Sciences, Vasile Goldis Western University of Arad, Romania

Email address: abrito@ff.ulisboa.pt

Background. Patients with triple negative breast cancer (BC) are prone to develop brain metastases, a condition with severe prognosis. To this fact, accounts the poor understanding of the mechanisms underlying brain metastases formation, which precludes the development of targeted therapies, and the absence of peripheral biomarkers that disqualifies timely disease detection. Here, we dissected BC cells and blood-brain barrier (BBB) endothelial cells molecular signatures and interplay along BC cells migration across brain microvasculature and brain colonization to uncover the players involved, searched for extracellular vesicles (EVs) and microRNAs (miRNAs) in plasma as biomarkers of the brain metastatic process, and analysed the brain expression of the deregulated circulating miRNAs to establish their brain origin.

Methods. Mouse were inoculated with triple negative BC cells in the carotid artery to induce preferential formation of metastases in the brain, and brain and plasma samples were examined along metastases development. Brain sections were examined by histologic, immunohistochemical and immunofluorescence analysis, as well as by *in situ hybridization*, and plasma samples were inspected by RT-qPCR, nanoparticle tracking analysis and flow cytometry.

Results. We observed an increasing presence of brain metastasis from 7 days onwards, with BC cells exhibiting an initial mesenchymal phenotype and migratory pattern, while epithelial markers increased along time, reflecting a mesenchymal-epithelial transition. They also presented proliferative features and expressed platelet-derived growth factor-B, β4-integrin and focal adhesion kinase, suggesting autocrine and/or paracrine regulation with adhesion signaling activation. Intercellular communication via gap junctions was clear among BC cells, and between BC and endothelial cells. Endothelial cells exhibited junctional and vesicular proteins alterations that, together with thrombin accumulation, reflect BBB compromise, whereas overexpression of pericyte markers indicates mural cells' activation. Augmented EVs, particularly of BBB origin, were associated with established metastases; contrarily, deregulated miRNAs in circulation were observed prior to brain metastases detection, and matched brain alterations, indicating their brain origin.

Conclusions. Our results provide in-depth understanding of BC brain metastasis formation, disclosing novel therapeutic targets. Moreover, they highlight miRNAs and EVs as biomarkers of metastases formation in early and advanced stages, respectively.

Work supported by FCT and the Champalimaud Foundation (Portugal), NKFIH (Hungary), and UEFISCDI (Romania).

OC2 - Comparison of SERCA and PMCA inhibition potential of polyoxotungstates

Aureliano, M.,[1,2*] Fraqueza, G., [2,3] Gumerova, N. I., [4] Rompel, A. [4] Cordoba-Granados, J.J.,[5] Berrocal, M. [5] and Mata, A. M. [5]

[1]FCT, Universidade do Algarve, 8005-139 Faro, Portugal; [2]CCMar, FCT, Universidade do Algarve, 8005-139 Faro, Portugal; [3]ISE, University of Algarve, 8005-139 Faro, Portugal; [4] Universität Wien, Fakultät für Chemie, Institut für Biophysikalische Chemie, 1090 Vienna, Austria; [5]Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura and Instituto de Biomarcadores de Patologías Moleculares, Universidad de Extremadura, 06006 Badajoz, Spain

*Correspondence: maalves@ualg.pt

Background. The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) and plasma membrane Ca^{2+}-ATPase (PMCA) are active Ca^{2+} transporters involved in several neurological diseases related to intracellular calcium dysregulation. Polyoxometalates (POMs) are well-known inhibitors of quite a few enzymes including SERCA and the Na^+/K^+-ATPase [1]. Herein, we compare for the first time the ability of several polyoxotungstates (POTs), for instance the mono-lacunary Wells-Dawson anion $[P_2W_{17}O_{61}]^{10-}$ (P_2W_{17}) and the Preyssler anion $[NaP_5W_{30}O_{110}]^{14-}$ (P_5W_{30}), to inhibit SERCA and PMCA pumps.

Methods. Polyoxometalates $Na_{12}[\alpha\text{-}P_2W_{15}V_3O_{56}]\cdot 24H_2O$ ($P_2W_{15}V_3$), $K_{10}[P_2W_{17}O_{61}]\cdot 20H_2O$ (P_2W_{17}) and $(NH_4)_{14}[NaP_5W_{30}O_{110}]\cdot 31H_2O$ (P_5W_{30}) were synthesized according to the published procedures and their purity was confirmed by IR and ^{31}P NMR spectroscopy. SERCA and PMCA activities were measured spectrophotometrically at 37 ˚C using the coupled enzyme pyruvate kinase/lactate dehydrogenase assay, as described elsewhere [1,2]. SH-SY5Y neuroblastoma cell cultures and treatments were performed as described in [3].

Results. The tested POTs strongly inhibited SERCA Ca^{2+}-ATPase activity, with the Preyssler being the most potent one, with an IC_{50} value of 0.4 μM. For Wells-Dawson POTs higher IC_{50} values were determined: 0.7 and 1 μM, respectively, P_2W_{17} and $P_2W_{15}V_3$. A mixed type of SERCA inhibition was observed for all POTs. The studied POTs showed to be stronger inhibitors of PMCA activity, compared to SERCA, once lower IC_{50} values where obtained for P_2W_{17}, P_5W_{30} and $P_2W_{15}V_3$, respectively, 0.1 (7 fold lower), 0.18 (2 fold lower) and 0.23 μM (4 fold lower) in comparion with SERCA. Besides, these compounds do not affect SH-SY5Y neuroblastoma cells viability at the IC_{50} values.

Conclusions. In sum, three POTs were tested as SERCA and PMCA inhibitors and showed higher activity against PMCA compared to SERCA. These POTs do not affect neuroblastoma cells. Therefore, this is interesting from the therapeutic point of view because they could be used to inhibits their targets (SERCA or PMCA) without affecting neuroblastoma cell survival.

References.

[1] Gumerova *et al*., *Metallomics*, **2018**,10, 287-295

[2] Salvador and Mata, *Biochem. J.*, **1996,** *315*, 183-187

[3] Berrocal *et al., Int. J. Mol. Sci.,* **2019**, 20, 3521

This research was funded by Grants from Fundação para a Ciência e a Tecnologia (FCT) project UIDB/04326/2020 (MA) and from the Spanish Ministerio de Economia y Competitividad (BFU2017-85723-P) to AM.

OC3 - Identification of germline mutations in patients with breast and difuse gastric cancer in Alentejo

Rui Dinis[1], Leoná Correia[2], Célia M. Antunes[3]

[1]Serviço de Oncologia, Hospital do Espírito Santo de Évora, Évora, Portugal; [2]Departmento de Química, Escola de Ciências e Tenologia, Universidade de Évora, Évora, Portugal; [3]Departmento de Ciências Médicas e da Saúde, Escola de Saúde e Desenvolvimento Humano, Universidade de Évora, Évora, Portugal;

Email address: ruidini@gmail.com

Background. Breast cancer and gastric cancer are defined as a serious public health problem, due to their high incidence and mortality. (1) The incidence of these diseases is influenced by both hereditary and environmental factors and are frequently underdiagnosed in the clinical practice. (2, 3). The objectives of this work were to identify the population carrying germline mutations of high penetrance genes for ductal, lobular and other breast cancer and CDH1 mutation in diffuse gastric cancer from the Oncology Service, HESE (Hospital do Espírito Santo de Évora, EPE), and to identify relevant germline mutations without high penetrance genes for breast cancer and the CDH1 gene in diffuse gastric cancer and lobular breast carcinoma.
Methods. The cohort was composed by women with triple negative breast cancer, HER2 positive or under 50 years of age, patients from the HESE and meeting NCCN criteria. Collection of data from clinical records: age, family history, histological classification of the tumor, degree of differentiation, mitotic index, staging, type of treatment and relapse status. DNA extraction from peripheral blood and identification of germline mutations was performed by NGS (cancer panel 4013).
Results. Of the 92 breast cancer patients, 14% had pathogenic germline mutations distributed among BRCA1, BRCA2, MUTYH, PTEN e PALB2, the majority being of BRCA2 (46%). The cohort of patients with BRCA 1 and BRCA2 mutations were on average 5 years younger (45 years vs 49 years), and more aggressive characteristics of the disease, characterized by a more advanced stage (majority stage II) compared to the group without mutations (majority stage I). In the 9 cases of diffuse gastric cancer, no pathogenic variants in the CDH1 gene were found.

Conclusions. In summary, hereditary breast cancer in this cohort is highly associated with BRCA2 mutations, concurring with others (4) and no pathogenic variants in the CDH1 gene were found, but the panel used did not contemplate for the CTNNA1 gene, recently associated with diffuse gastric cancer syndrome (5). In conclusion, this work may contribute to optimize the criteria used for the screening and early diagnostics.

References

(1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7-30. doi: 10.3322/caac.21590. Epub 2020 Jan 8. PMID: 31912902.
(2) McDonald, E., Clark, A., Tchou, J., et al. Clinical Diagnosis and Management of Breast Cancer. J Nucl Med. 57: 9S-16S. (2016).
(3) Guilford P, Humar B, Blair V. Hereditary diffuse gastric cancer: translation of CDH1 germline mutations into clinical practice. Gastric Cancer. 2010;13(1):1–10.
(4) Antoniou AC, Easton DF. Models of genetic susceptibility to breast cancer. *Oncogene* 2006; 25: 5898–5905.
*(5) Clark, D.F., Michalski, S.T., Tondon, R. et al. Loss-of-function variants in CTNNA1 detected on multigene panel testing in individuals with gastric or breast cancer. Genet Med **22,** 840–846 (2020). https://doi.org/10.1038/s41436-020-0753-1*

OC4 - Peroxiporins and pancreatic cancer: new roles for aquaporin-3 and aquaporin-5 in tumor biology

Inês V. da Silva[1], Patrícia M. Silva[2], Nuno C. Santos[2], Graça Soveral[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal,
[2]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Email address: imsilva1@campus.ul.pt

Background. Aquaporins (AQPs) are transmembrane protein channels responsible for the bidirectional transfer of water and small solutes across cell membranes. Being involved in a wide range of physiological functions and diseases [1], AQPs have great potential for pharmacologic targeting and drug discovery. Recent studies showed that the altered expression of several AQPs is associated with various cancers, though the underlying molecular mechanism remains unclear. The overexpression of AQP3 and AQP5 was detected in pancreatic ductal adenocarcinoma cells [2], being postulated their association with cell migration and proliferation, angiogenesis and overall tumor formation [3].

Methods. In this work, we aimed at evaluating the contribution of AQP3 and AQP5 as water, glycerol and/or hydrogen peroxide channels to the settings of tumor formation and development using a loss-of-function strategy. BxPC3 cells, an in vitro model of pancreatic ductal adenocarcinoma that expresses high levels of AQP3 and AQP5, were silenced for AQP3 and/or AQP5 expression. Silenced cells were evaluated for their AQP3 and AQP5 expression levels, membrane permeability for water, glycerol and hydrogen peroxide, impact on cell migration, as well as effect on several markers of tumor (ERK1/2, EGFR, c-Fos, c-Jun,) and cell differentiation (Ecad and Vim).

Results. Our results confirmed that AQP3 and AQP5 have impact on cell membrane permeability to glycerol and hydrogen peroxide, which corroborates their activity as aquaglyceroporin (AQP3) and peroxiporins (AQP3 and AQP5). AQP3 and AQP5-silenced cells showed slower recover of the wounded area (around 10% and 15%, respectively, comparing to controls) evidencing the association between these isoforms and cell migration. Moreover, AQP3 and AQP5-silenced cells express lower levels of ERK1 (0.5- and 0.7-folds, respectively), ERK2 (0.4- and 0.8-folds) EGFR (0.6-folds for AQP3), c-Fos (0.3- and 0.5-folds) and c-Jun (0.1- and 0.7-folds), as well as E-cad (0.6-folds for AQP3) and Vim (0.2- and 0.6-folds).

Conclusions. Our results reveal a correlation between AQP3 and AQP5 expression and the induction of key players of the EGFR/ERK/p38 MAPK signalling pathway and epithelial mesenchymal transition (EMT), suggesting the involvement of peroxiporins in cancer initiation and progression, and highlighting their importance in tumor biology. These findings may foster new strategies towards the development of antitumoral therapies.

References.

[1] Soveral G, Nielsen S, Casini A. (Eds). (2016) Aquaporins in Health and Disease: New Molecular Targets for Drug Discovery. Boca Raton; London; New York: CRC Press, Taylor & Francis Group.
[2] Direito I, Paulino J, Vigia E, Brito MA, Soveral G. (2017) Differential expression of aquaporin-3 and aquaporin-5 in pancreatic ductal adenocarcinoma. J Surg Oncol. 115(8):980-996.
[3] Marios C. Papadopoulos, Samira Saadoun (2015) Key roles of aquaporins in tumor biology, BBA. 1848(10):2576-2583.

OC5 - The RNA-binding protein PNPase is a novel regulator of biofilm formation and virulence in *Listeria monocytogenes*

Quendera, A.P.[1], Pinto, S.N.[2], Antunes, W.[3], Arraiano, C.M.[1] and Andrade, J.M.[1]

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Avenida da República 2780-901, Oeiras, Portugal. [2]Institute for Bioengineering and Biosciences (IBB) and Associate Laboratory - Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal. [3]Laboratório de Imagem, Nanomorfologia e Espectroscopia de Raios-X (Linx) da Unidade Militar Laboratorial de Defesa Biológica e Química (UMLDBQ), Instituto Universitário Militar, Centro de Investigação, Inovação e Desenvolvimento da Academia Militar, Av. Dr Alfredo Bensaúde, 1100-471 Lisboa, Portugal.

Presenting author: aquendera@itqb.unl.pt

Background. Bacterial biofilms provide a stress-enduring environment that makes bacteria more resilient to both the immune system and antibiotics. Accordingly, biofilm-related diseases are typically persistent infections, and a challenge for medical treatment. The knowledge of novel biofilm regulators may contribute to develop new strategies to fight microbial infections.

Methods. We used biochemical, biophysical, and microbiology tools to study the role of the RNA-binding protein polynucleotide phosphorylase (PNPase), a well-known RNA nucleolytic enzyme, in the human pathogen *Listeria monocytogenes*. Biofilms from the mutant and wild-type strains were characterized by biomass production, followed by extraction and quantification of the matrix composition, and analysis by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) techniques. Moreover, we analysed the requirement of PNPase in the infection of human cell lines.

Results. Here we show that inactivation of *Listeria* PNPase leads to strong defects in biofilm production, and strikingly, affects biofilm morphology.

1. We demonstrate that PNPase is a previously unrecognized regulator of biofilm matrix composition, greatly affecting the levels of proteins, sugars, and extracellular DNA. The reduction in all these components of the extracellular polymeric substance (EPS) correlates with a less thick biofilm found in PNPase-deficient bacteria, as confirmed by different microscopy analysis.

2. As result, we found that the less stable and less resistant biofilm produced in the PNPase mutant is more susceptible to antibiotic treatment, which contributes for a more efficient biofilm eradication.

3. Furthermore, infection assays in different eukaryotic cell lines confirmed that PNPase deletion leads to the severe attenuation of *Listeria monocytogenes* pathogenicity.

Conclusions. Overall, our results show that PNPase is a novel regulator of biofilm formation and human cellular invasion of a bacterial pathogen. This work presents PNPase as a new and attractive target for the control of bacterial infection and highlights the expanding role of RNA-binding proteins as critical players in pathogenicity.

OC6 - The role of sacsin in the pathological aggregation of intermediate filaments

Migueis M[1], Murtinheira F[2], Rodrigues MS[3], Vitorino M[4], Herrera F[5]

[1]Laboratório de estrutura e dinâmica celular, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal; [2]BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal.

Email address: mafalda.migueis1998@gmail.com

Background. Sacsin is a very large protein encoded in humans by the SACS gene. Loss-of-function mutations in this gene cause autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), a rare ataxia characterized by atrophy of the anterior cerebellar vermis associated with Purkinje cell death. Its clinical phenotype is characterized by increased muscle tone, difficulty coordinating movements, distal muscle wasting, involuntary eye movement and speech difficulties. Currently, there is no available therapy for ARSACS. Moreover, the loss of sacsin causes alterations in the expression and distribution of intermediate filaments in neurons, which change the architecture and mechanical properties of cells and tissues. However, it was observed in our laboratory that glial cells also express sacsin, and its removal in astroglial-like cells caused alterations in intermediate filaments. This observation suggests a possible role of glial cells in ARSACS and would explain non-neural features of this disorder. Therefore, we are interested in elucidating the mechanisms by which sacsin loss leads to IF collapse and agregation in N9 microglial cells.

Methods. We deleted sacsin from N9 rat microglial cells by means of a CRISPR/Cas9 approach, isolated sacsin knockout cell lines with Flow Cytometry-assisted Cell Sorting (FACS), and analyzed the effects of sacsin loss in the expression and distribution of the intermediate filament network and other cellular structures and functions. The intermediate filament expression and distribution profile of sacsin$^{-/-}$ N9 microglial cells was characterized by means of immunocytochemistry, western blotting, widefield and confocal fluorescence microscopy, and atomic force microscopy (AFM). This last method was used to detect the alterations of mechanical and viscoelastic properties of knockout cells, caused by the disorganization of the cytoskeleton. We are currently doing a tentative screening on the possible mechanisms regulating sacsin functions or preventing the consequences of its loss of function in ARSACS. This screening is based on other cellular parameters changes, such as basal oxidative stress, resistance to exogenous stress and toxins, mitochondrial activity, and ATP levels.

Results. Rat microglia expressed vimentin and nestin, and high levels of sacsin. Removal of sacsin caused alterations in the structure of intermediate filament networks similar to those found in astroglial-like cells. By AFM, N9 knockout cells had their viscoelastic and mechanical proprieties affected. It was necessary to apply a weaker force to deform the knockout cells in comparison to the control cells, since their cytoskeleton was already disarrayed.

Conclusions. We acquired a better understanding of the mechanisms involved in sacsin functions, with especial focus on its effect on intermediate filaments. Being sacsin a protein that regulates neuronal intermediate filament assembly and intracellular distribution, the knockout of this protein in the cell will lead to the collapse of intermediate filaments and the consequent cytoskeleton disorganization, studied in previous experiments. This hypothesis was observed in our results, where sacsin$^{-/-}$ N9 microglial cells had alterations in their intermediate filament profile and mechanical properties. Our results could be relevant for the treatment of ARSACS, but also for a wide spectrum of human pathologies caused by disruption of the intermediate filament networks.

SYMPOSIUM 2 – PLANT BIOLOGY AND BIOTECHNOLOGY

With an expected world population of almost 10 billion by 2050, and increasingly harsh conditions to agricultural production due to climate change, significant advances in plant sciences are needed to cope with global food security. A more comprehensive understanding of the interactions between plant, environmental conditions and cultural practices is needed. To achieve this goal, a better integration of knowledge at different levels, from molecular biology to whole-plant physiology, is required. Within this session, we will get aware on the diversity of current approaches to in plant sciences, from new breeding techniques to high-throughput plant phenotyping or plant ecological networks.

IL3
Elizabete Carmo-Silva (Lancaster Univ., UK)

IL4
Helena Carvalho (CIBIO-UP)

INVITED LECTURES S2

(IL3-IL4)

Elizabete Carmo-Silva *Lancaster Environment Centre, Lancaster University*	Rubisco activase isoform diversity and crop adaptation to dynamic environments
Helena Carvalho *University of Porto - Research Center in Biodiversity and Genetic Resources*	Unravelling the Function of Glutamine Synthetase of the Prokaryotic Type (GSI-like) in Nitrogen Signalling in Medicago truncatula

Chairs: Jorge M. Silva (FCUL); Teresa Lino-Neto (UMinho)

IL3 - Rubisco activase isoform diversity and crop adaptation to dynamic environments

Elizabete Carmo-Silva

Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

Email address: e.carmosilva@lancaster.ac.uk

Rubisco plays a central role in photosynthesis. It is an essential, yet imperfect enzyme, and frequently limits carbon assimilation in crops. Regulation of Rubisco activity in plants depends on interaction with multiple cellular components and is continuously adjusted in response to environmental fluctuations. Rubisco is prone to inhibition by the tight-binding of sugar-phosphate derivatives to catalytic sites. ATP-dependent removal of such inhibitors by Rubisco activase (Rca) is regulated in response to changes in light and temperature

IL4 - Unravelling the Function of Glutamine Synthetase of the Prokaryotic Type (GSI-like) in Nitrogen Signalling in *Medicago truncatula*

Seabra A[1], Silva L[1], Carvalho H[1]*

[1]University of Porto - Research Center in Biodiversity and Genetic Resources (CIBIO-InBio), Rua Padre Armando Quintas, nº 7.4485-661, Vairão. Portugal

*Email address: helenacarvalho@cibio.up.pt

Nitrogen (N) is a key element for plant growth and development, but also a metabolic signal that is sensed and transduced by plants. The identification of the molecular players of the N-mediated signalling events in plants is absolutely necessary to fully understand the N-regulatory networks, and ultimately to provide the knowledge necessary to improve N-use efficiency (NUE). We have recently identified novel class of molecular players (GSI-like proteins) in *Medicago truncatula* and obtained strong evidence pointing to an involvement of these molecules in nitrogen-signalling pathways known to influence developmental processes such as lateral root and root nodule formation. This presentation is intended to provide an overview of the distribution of genes encoding glutamine synthetase of the prokaryotic type (GSI-like) across the plant kingdom and focus on the characterization of the entire *M. truncatula* GSI-like gene family and its encoded proteins.

Although GSI-like genes are widespread in plants, its exact function remains unidentified. In the great majority of plants, these genes encode a fusion protein, referred to as NodGS, composed of a C-terminal GSI-like domain and an N-terminal amidohydrolase domain homologous to nodulin 6. However, genes encoding proteins with a single GSI-like domain are exceptionally found in *Medicago truncatula* and few other plant species. The genome of *M. truncatula* contains three genes encoding single-domain GSI-like proteins (*MtGSIa, MtGSIb* and *MtGSIc*), and two additional genes encoding NodGS proteins (*MtNodGSa* and *MtNodGSb*). The genes are expressed *in planta*, but none of the *M. truncatula* GSI-like proteins retain GS activity and thus its function is not related to glutamine synthesis. However, the conservation of GSI-like genes across all plant species suggests an important, but yet unknown function for the encoded proteins. We characterized the entire *GSI-like* gene family *of M. truncatula,* localized the expression of the individual genes using promotor-*gusA* fusions in the homologous transgenic system, studied the responses to nitrogen and hormone signals, produced recombinant proteins in *E. coli* and investigated the functionality of the purified proteins. Taken together, our results indicate a function related to nitrogen signalling, but the two classes of GSI-like proteins (GSI and NodGS) appear to perform different physiological functions. During this presentation we will provide evidence supporting these assumptions and will discuss the possible role of GSI-like proteins within the N-signalling networks in *M. truncatula*.

ORAL COMMUNICATIONS S2

OC7	Rita Abranches	Expression of recombinant cardosin B in tobacco BY2 cells: an alternative system for the production of active milk clotting enzymes
OC8	Ana Rita Cavaco	Fatty acid profiling as a chemotaxonomic tool and biomarker for grapevine tolerance/susceptibility to Plasmopara viticola
OC9	Pedro Correia	High-throughput phenotyping of wheat physiological and metabolic responses to high temperature and drought
OC10	Joana Figueiredo	Inside proteomics: uncovering cultivar specific grapevine apoplast dynamics
OC11	Marisa Maia	The search for grapevine disease-associated biomarkers: combining metabolomics with targeted gene expression

Chairs: Jorge M. Silva (FCUL); Teresa Lino-Neto (UMinho)

OC7 - Expression of recombinant cardosin B in tobacco BY2 cells: an alternative system for the production of active milk clotting enzymes

André Folgado[1], Rita Abranches[1]

[1]Plant Cell Biology Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, ITQB NOVA, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

Email address: ritaa@itqb.unl.pt

Background: *Cynara cardunculus* L. or cardoon is a plant that is used as a source of milk clotting enzymes during traditional cheese manufacturing. This clotting activity is due to aspartic proteases (APs) found in the cardoon flower, named cyprosins and cardosins. APs from cardoon flowers display a great degree of heterogeneity, resulting in variable milk clotting activities and directly influencing the final product. Producing these APs using alternative platforms such as bacteria or yeast has proven challenging, which is hampering their implementation on an industrial scale.

Methods: We have generated transgenic tobacco BY2 cell lines producing cardosin B from *C. cardunculus*, including a line in which the recombinant Cardosin B is fused to a red fluorescent protein.

Results: These cultures successfully produced active cardosin B and a downstream purification process pipeline was developed to obtain pure cardosin B. The purified enzyme displayed proteolytic activity towards milk caseins and milk clotting activity under standard cheese manufacturing conditions. We also identified an unprocessed form of cardosin B and further investigated its activation process. The use of protease-specific inhibitors during the activation process suggested a possible role of a cysteine protease in cardosin B processing. Mass spectrometry analysis identified three cysteine proteases containing a granulin-domain as candidates for cardosin B processing.

Conclusions: Our findings suggest a possible interaction between aspartic and cysteine proteases and contribute to expand the understanding of the mechanisms underlying the regulation and processing of plant APs. This work also paves the way for the use of tobacco BY2 cells as an alternative production system for active cardosins and represents an important advancement towards the industrial production of cardoon APs.

References: Folgado A, Abranches R. (2021). Tobacco BY2 cells expressing recombinant cardosin B as an alternative for production of active milk clotting enzymes. Scientific Reports 11, 14501.

OC8 - Fatty acid profiling as a chemotaxonomic tool and biomarker for grapevine tolerance/susceptibility to *Plasmopara viticola*

Cavaco AR[1], Laureano G[1], Matos AR[1], Figueiredo A[1]

[1]Biosystems and Integrative Sciences Institute (BioISI), Faculty of Science, University of Lisbon, Lisboa, Portugal

Email address: arcavaco@fc.ul.pt

Background. Grapevine (*Vitis vinifera* L.) is one of the most valuable crops worldwide. Wine industry plays a key role in many countries economy, where Portugal is within the top ten producers. *V. vinifera* premium cultivars used for wine and table grape production are prone to several diseases, being downy mildew, caused by *Plasmopara viticola*, one of the greatest threats to modern viticulture. In most vineyards, disease control strategies rely on the application of high fungicide amounts every year throughout the entire grapevine developing process, raising concerns at environmental, economic and human health levels. Therefore, the main priority in today's agriculture is reducing phytochemicals usage. In order to uncover and promote sustainable disease control strategies, identifying molecular mechanisms of resistance and biomarkers is vital. Lipids and fatty acids play crucial roles in plant immunity, which have been highlighted over the past few decades. An increasing number of studies show that these molecules are pivotal in the interaction with pathogens. The roles played by plant lipids fit in a wide spectrum from the first physical barrier against pathogens, the cutin, to the signaling pathways that trigger different immune responses and defense related genes. Hence, the basal levels of these molecules may be crucial to distinguish tolerant and susceptible grapevine varieties and species.

Methods. Several field grown grapevine genotypes displaying different tolerance degrees to pathogens were analysed. Leaf fatty acid contents and composition was analyzed by gas chromatography. Moreover, a separation and analysis of the different classes of lipids was also performed by thin layer chromatography followed by gas chromatography. Since membrane fluidity is affected by its lipid composition, namely the unsaturation degree of their fatty acids, expression analysis of fatty acid desaturase genes was also conducted by quantitative real time PCR.

Results. A clear separation between tolerant and susceptible accessions was observed. A higher α-linolenic to linoleic acids ratio was found in the tolerant genotypes, while the double bond index was higher in susceptible cultivars. The lipid analyses of four selected accessions, two susceptible and two tolerant to *P. viticola*, shows that monogalactosyldiacylglycerol and neutral lipids allow a discrimination between the two groups. A principal component analysis of the levels of these lipids indicates that monogalactosyldiacylglycerol may be a biomarker for susceptibility to *P. viticola*, while higher levels of neural lipids (such as di- and triacylglycerols) may be a marker for tolerance.

Conclusions. Our results highlight for the first time fatty acid ratios and lipid class relative abundances as chemotaxonomic candidates to assess susceptibility and tolerance of *V. vinifera* cultivars and *Vitis* species. The observed differences in fatty acid profiles are mainly associated to membrane fluidity characteristics, which arise as the core differentiating feature between tolerance and susceptibility. The obtained data indicates a promising path leading to an early screening for tolerance biomarkers in grapevine breeding programs.

OC9 - High-throughput phenotyping of wheat physiological and metabolic responses to high temperature and drought

Pedro M. P. Correia[1], Jesper Cairo Westergaard[2], Gemma Molero[4], Anabela Bernardes da Silva[1], Thomas Roitsch[2,3], Elizabete Carmo-Silva[5], Jorge Marques da Silva[1]

[1]Universidade de Lisboa, BioISI, Portugal, [2]Copenhagen University, Denmark, [3]Global Change Research Institute, Czech Republic, [4]CIMMYT, Mexico, [4]University of Lancaster, LEC, United Kingdom

Email address: pmpcorreia@fc.ul.pt

Nearly 40% of the global wheat yield fluctuations are explained by climatic variation, being heatwaves and drought among the top stressors. This work aimed to optimise high-throughput methods to phenotype wheat plants under drought and heat stress; identify phenotypic traits that reflect stress responses; characterise and classify 10 wheat genotypes by growth dynamics, and ultimately understand the regulatory mechanisms on the primary carbohydrate metabolism under these stress conditions. Wheat plants were grown in a fully automated plant facility under 25/18ºC day/night for 25 days, and then the temperature was increased for seven days (38/31ºC day/night) while maintaining half of the plants well irrigated and half at 30% field capacity. Multispectral (10 discrete bands in the range 365-970 nm) and thermal images, and pot weights were registered twice daily. At the end of the experiment, the main carbohydrates and the activity of key carbohydrate enzymes were quantified. Regression machine learning models were successfully established to predict plant biomass by image-extracted parameters. Leaf temperature and evapotranspiration traits expressed significant genotype-environment interactions (GxE) at the end of the experiment. Adaptation to these stresses included changes in the carbohydrate metabolism, particularly in the sucrolytic and the glycolytic pathways. The observed genetic differences in sensitivity to high temperature and extended drought can be exploited to improve wheat resilience to climate change.

Acknowledgements: Financial support by UIDB/04046/2020 and UIDP/04046/2020 Centre grants from FCT (BioISI), FCT research project INTERPHENO (PTDC/ASP-PLA/28726/2017) and Access to Research Infrastructures activity in the Horizon2020 Programme of the EU (EPPN2020 Grant Agreement 731013) is gratefully acknowledged. Pedro Correia acknowledges FCT(Portugal) for the financial support via a fellowship from BioSys PhD program PD65-2012 (Ref SFRH/PD/BD/130973/2017).

OC10 - Inside proteomics: uncovering cultivar specific grapevine apoplast dynamics

Figueiredo J. [1,2,3], Cavaco A.R. [1], Guerra-Guimarães L. [4,5], Leclercq C.C. [6], Santos R.B. [1], Silva M.C. [4,5], Renaut J. [6], Sousa Silva M. [2,3], Figueiredo A. [1]

[1]BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal; [2]Laboratório de FTICR e Espectrometria de Massa Estrutural, Faculdade de Ciências da Universidade de Lisboa, Portugal; [3]Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Portugal; [4]Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal; [5]Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal; [6]Luxembourg Institute of Science and Technology, Belvaux, Luxembourg

Email address: jffigueiredo@fc.ul.pt

Background. Plant apoplast, the cellular compartment external to the plasma membrane including the cell wall, is particularly demanding to analyse. Despite our knowledge on apoplast involvement on several biological processes, its composition and dynamics is still poorly known due to the lack of efficient extraction processes, particularly considering woody plants as grapevine. Grapevine (*Vitis vinifera* L.) is among the most important fruit crops worldwide, with an enormous economic impact. *V. vinifera* premium cultivars are highly susceptible to pathogens, particularly to fungi and oomycete. The apoplast is considered as the first battlefield in plant-pathogen interactions and a deeper knowledge of its composition is crucial to understand the different dynamics of tolerant and susceptible genotypes. In a first approach to grapevine apoplast, we have developed an optimized vacuum-infiltration-centrifugation (VIC) method that allows a simultaneous extraction of grapevine apoplastic proteins and metabolites. This methodology was applied to study two grapevine cultivars with different tolerance to pathogens, namely to *Plasmopara viticola* (downy mildew disease).

Methods. *V. vinifera* cv. 'Trincadeira' and 'Regent', two grapevine cultivars with different phenological characteristics and tolerances to *P. viticola* were used for APF proteome characterization. APF proteins were extracted through a VIC-based optimized method and its quality was accessed by MDH activity. APF proteome was further analysed by nanoLC-MS/MS [1].

Results. At a constitutive level, 721 proteins were detected being common to both grapevine cultivars. Around 90% of the identified APF proteins were further validated as secreted proteins by several bioinformatic tools (using the classical or unconventional secretory pathway). A functional categorization of these proteins indicated that they are mostly involved in cell wall metabolism, protein metabolism and response to stress.

Conclusions. The proposed methodology opens new insights for global characterization of grapevine APF, searching compartment complexity and paving the way to uncover signalling networks and interactions within a systems biology approach. Further APF analysis during grapevine-*P. viticola* interaction will allow a deeper understanding of the molecular mechanisms involved in the direct communication between both plant and pathogen, and uncover the plant defence and the pathogen infection strategies.

References: [1] Figueiredo, J., *et al.* (2020) An apoplastic fluid extraction method for the characterization of grapevine leaves proteome and metabolome from a single sample. *Physiologia Plantarum*. doi.org/10.1111/ppl.13198

Acknowledgments: Portuguese Foundation for Science and Technology (FCT, Portugal) funded fellowships and contracts to AF, MSS, ARC and JF: IF/00819/2015, CEECIND/02246/2017, SFRH/BD/131030/2017, SFRH/BD/137066/2018, respectively. FCT funded the Research Units and project: BioISI (UIDB/04046/2020 and UIDP/04046/2020), and LEAF (UID/AGR/04129/2019), and the project PTDC/BIA-BQM/28539/2017.

OC11 - The search for grapevine disease-associated biomarkers: combining metabolomics with targeted gene expression

Maia M[1,2], Ferreira AEN[1], Nascimento R[2], Monteiro F[3,4], Traquete F[1], Marques AP[1], Cunha J[5], Eiras-Dias JE[5], Cordeiro C[1], Figueiredo A[2] and Sousa Silva M[1]

[1]Laboratório de FTICR e Espectrometria de Massa Estrutural, Faculdade de Ciências, Universidade de Lisboa, Portugal; [2]Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Portugal; [3]Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Portugal; [4]Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Portugal; [5]Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Portugal.

Email address: mrmaia@fc.ul.pt

Background. *Vitis vinifera*, one of the most cultivated fruit crops, is susceptible to several diseases particularly caused by fungal and oomycete pathogens. In contrast, other *Vitis* species (American and Asian) display different degrees of tolerance/resistance to these pathogens, being widely used in breeding programs to introgress resistance traits in elite *V. vinifera* cultivars. Secondary metabolites are important players in plant defense responses. The characterization of the metabolic profiles associated with disease resistance and susceptibility traits in grapevine is a promising approach to identify trait-related biomarkers. A combined approach based on untargeted metabolomics and targeted gene expression analysis was used to identify putative biomarkers in susceptible and resistant/tolerant genotypes.

Methods. Eleven *Vitis* genotypes with different resistance degrees to pathogens were analyzed. Metabolite extraction was performed as previously described[1] and the extracts were analyzed by direct infusion on a FT-ICR mass spectrometer. Spectra were acquired in both electrospray ionization modes (ESI+ and ESI−). Statistical analysis was applied to investigate the metabolic profile similarities between *Vitis* samples. Discriminatory peaks were annotated and mapped into metabolic pathways. Genes encoding biosynthetic or catabolic enzymes related to discriminatory metabolites were selected and their expression was evaluated by qPCR. For qPCR normalization, reference genes (RGs) for the constitutive analysis of grapevine genotypes were established for the first time.

Results. A separation trend between wild *Vitis* and *V. vinifera* cultivars metabolomes was observed. 190 unique masses with significant variation between our comparison groups were putatively annotated. Compounds were mapped into metabolic pathways being flavonoid biosynthesis and flavone and flavonol biosynthesis the most discriminatory pathways. 7 genes were selected for expression analysis and 3 RGs were defined and used for gene expression normalization. *LAR2 (*involved in catechin biosynthetic pathways) presented a higher expression in the susceptible plants group, in agreement with catechin accumulation.

Conclusions. The leaf metabolome of 11 *Vitis* genotypes was characterized and several compounds were selected as promising biomarkers. We propose that both catechin/epicatechin and LAR2 may be putative biomarkers of susceptibility to fungal and oomycete pathogens[2].

References.

1. M. Maia, F. Monteiro, M. Sebastiana, A. P. Marques, A. E. N. Ferreira, A. P. Freire, C. Cordeiro, A. Figueiredo, and M. Sousa Silva, *EuPA Open Proteom*, **2016**, *12*, 4 (DOI:10.1016/j.euprot.2016.03.002).
2. M. Maia, A. E. N. Ferreira, R. Nascimento, F. Monteiro, F. Traquete, A. P. Marques, J. Cunha, J. E. Eiras-Dias, C. Cordeiro, A. Figueiredo, and M. Sousa Silva, *Scientific Reports*, **2020**, *10*, 15688 (DOI: https://doi.org/10.1038/s41598-020-72781-2).

SYMPOSIUM 3 - TOXICOLOGY AND ENVIRONMENTAL BIOCHEMISTRY

Toxin-poison and other bioactive substances, whether chemically synthesized or produced by living organisms, such as bacterial toxins, mycotoxins, phycotoxins or zootoxins, allergens or environmental pollutants may trigger a number of responses within the biochemical machinery of the organisms, unleashing diverse AOPs. The exposome is a determinant key for many undesired outcomes.
The session will cover several components of the exposome, their target biomolecules, cell responses and biomarkers, the development of new detection methods for toxins, allergens and pollutants as well as the development of mitigation strategies.

IL5
Ana Catarina Sousa (UÉv)

IL6
Maria João Bebbiano (UAlg)

INVITED LECTURES S3

(IL5-IL6)

Ana Catarina Sousa
Department of Biology, School of Sciences and Technology, University of Évora; Comprehensive Health Research Centre , University of Évora; Center for Marine Environmental Studies, Ehime University, Japan

Hormones messed up - a tale of endocrine disruptors in the XXI century

Maria João Bebianno
Centre for Marine and Environmental Research (CIMA), University of Algarve

Are micro and nanoplastics toxic?

Chairs: Ana R. Costa (UÉv); Jose Maria Manzano (TUM, Germany)

IL5 - Hormones messed up - a tale of endocrine disruptors in the XXI century

Ana Catarina Sousa[1,2,3], M. Ramiro Pastorinho[2,4], Sónia D. Coelho[5], Tomohiko Isobe[6], Rumi Tanoue[3], Tatsuya Kunisue[3], Shinsuke Tabane[3]

[1]Department of Biology, School of Sciences and Technology, University of Évora, Portugal; [2]Comprehensive Health Research Centre (CHRC), University of Évora, Portugal; [3]Center for Marine Environmental Studies (CMES), Ehime University, Japan; [4]Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Portugal; [5]Department of Biology and CESAM-Centre for Environmental and Marine Studies, University of Aveiro, Portugal; [6]Center for Environmental Health Sciences, National Institute for Environmental Studies, Japan

Email address: acsousa@uevora.pt

The last century was a stage for a dramatic change in population growth, economic development, environmental degradation and climate change, with great implications for human health. The extensive advances in the prevention and treatment of disease lead to a marked decrease in child mortality and transmissible diseases and to an increase in longevity. However, such accomplishments were followed by a marked increase in the incidence of non-communicable diseases (NCDs) which are now the leading cause of death globally, killing more people each year than all other causes combined. The prevalence of NCDs alongside with an ageing population creates an urgency to implement effective prevention strategies; if not, the future costs with healthcare systems will be unaffordable. However, in order to implement prevention programs, it is mandatory to clearly understand which factors are affecting human health.

Today there are no doubts that human health and particularly NCDs, are associated with the increasing exposure to environmental contaminants. Some of these contaminants have the ability to mimic or antagonize the action of hormones, thus affecting the normal functioning of organisms leading to widespread effects in humans and wildlife. These chemicals, defined as endocrine disruptors (EDCs) are ubiquitous in the environment. Considering the amount of these compounds released into the environment and present in everyday modern life, namely in consumer products, wildlife and humans are constantly exposed and, therefore, subjected to their deleterious effects. Available studies from animal models, human clinical observations, and epidemiological studies converge to implicate EDCs as a significant concern to public and ecosystems health. Growing evidence suggests that EDCs are involved in several cancer types, autoimmune diseases and allergies, infertility and malformations of newborn male genitalia, neurodevelopmental diseases, type 2 diabetes and other metabolic disorders. Some of these pathologies as, for example, obesity, are of major concern in modern societies being already classified as epidemics.

In this presentation, an overview of the most relevant endocrine disruptors will be provided alongside with the characterization of exposure sources and pathways, so that measures to prevent exposure can be implemented. Particular emphasis will be given to the studies reporting EDCs levels in Portugal.

IL6 - Are micro and nanoplastics toxic?

Maria João Bebianno

Centre for Marine and Environmental Research (CIMA), University of Algarve, Campus de Gambelas, 8000-397 Faro, Portugal.

Email address: mbebian@ualg.pt

Plastic contamination in the marine environment has been a cause of concern all over the world, representing the majority (around 80%) of marine litter. Marine litter comes from land-based and ocean sources. From land-based sources, plastics result from poor waste management practices and is estimated that every year one million tons of plastic enter the ocean from rivers. In the ocean, the main sources are maritime transport, industrial exploration and, abandoned or otherwise discarded fishing gears. The majority is found in coastal areas but also in ocean gyres where they can be found in shallow waters to the deep-ocean. Primary microplastics enter the ocean as micro or nanoplastics while secondary microplastics and nanoplastics result from the weathering of single-use plastics. One of the major concerns of the presence of micro and nanoplastics in the marine environment is the possibility of these plastic particles being able to adsorb other stressors such as metals and organic compounds and become a source of these stressors to marine species.

The aim of this presentation is to show the impact of the presence of microplastics in several species from the South Coast of Portugal and highlight the effects of virgin micro and nanoplastics in marine bivalves as well as their effects when organic contaminants are adsorbed.

Aknowledgements. This work funded by the EPHEMARE and RESPONSE projects, (FCT JPIOCEANS/0005/2015 and JPIOCEANS/0005/2020. We further acknowledge FCT for the funds attributed to CIMA, University of Algarve UID/00350/2020.

ORAL COMMUNICATIONS S3

OC12	Patrícia Alves	Effects of cannabidiol and delta-9-tetrahydrocannabinol on placental extravillous trophoblast cells migration
OC14	Alexandra Penha	Identification of the acarological distribution using household dust collected in Coastal regions of Portugal: Aveiro as a case study
OC15	João Lopes	Differential effect of ABTS on the laccase and peroxidase-catalyzed degradation of organic pollutants
OC16	Ramiro Pastorinho	Suspension and deposition patterns of microplastics from a 3D printing source

Chairs: Ana R. Costa (UÉv); Jose Maria Manzano (TUM, Germany)

OC12 - Effects of cannabidiol and delta-9-tetrahydrocannabinol on placental extravillous trophoblast cells migration

Alves P[1,2], Amaral C[1,2], Teixeira N[1,2], Correia-da-Silva G[1,2]

[1]UCIBIO-REQUIMTE, Biological Sciences Department, Laboratory of Biochemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal; [2]Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal

Email address: patriciaalves.95@hotmail.com

Background. The use of medical cannabis and cannabinoid-based products has been increasing in the last years. It is known that cannabidiol (CBD), a non-psychotropic cannabinoid from *Cannabis sativa*, impairs placental drug transport. Moreover, we have already reported that CBD interferes in endometrial cells decidualization and that delta-9-tetrahydrocannabinol (THC), the main phytocannabinoid, induces apoptosis and disrupts endocrine function of placental trophoblast cells. However, there is still much to unveil concerning the impact of cannabinoid mixtures on reproductive health. Thus, the aim of this work was to evaluate the impact of CBD, THC and of the combination of CBD plus THC (1:1), in trophoblast function, namely in the migration of extravillous trophoblast cells (EVTs), using the HTR-8/SVneo cell line, a widely used *in vitro* model obtained from first trimester placenta.

Methods. HTR-8/SVneo cells (ATCC, USA) were treated with CBD, THC and CBD+THC (1:1), at 2 μM, for 24 and 48 h. Their effects on cell migration were studied through Wound healing assay and by assessing MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNA levels (qRT-PCR). The activity levels of MMP-2 and -9 were evaluated by Gelatin zymography. Alterations on the activation of ERK 1/2 signalling pathway were assessed by Western blotting.

Results. CBD alone induced a significant ($p < 0.01$) reduction of wound closure percentage, which was enhanced by the combination of CBD plus THC ($p < 0.001$), when compared with the control. CBD significantly increased the expression of *MMP-2*, *MMP-9* ($p < 0.001$), *TIMP-1* ($p < 0.05$) and *TIMP-2* ($p < 0.01$) genes, which was also observed in CBD+THC combination for MMP-2, MMP-9 ($p < 0.001$) and TIMP-2 ($p < 0.05$) genes. CBD increased MMP-2 ($p < 0.05$) and MMP-9 ($p < 0.001$) activity, whilst the combination decreased it. THC alone had no effects. CBD also increased the activation of ERK signalling pathway ($p < 0.05$), which was not observed in THC treated cells with or without CBD.

Conclusions. This work demonstrates that the consumption of cannabinoid-based products containing either CBD alone or combined with THC during pregnancy interferes in EVTs migration. Therefore, this crucial phase of placental development may be affected, compromising pregnancy success.

Acknowledgments. The authors thank to Fundação para a Ciência e Tecnologia (FCT) for P. Alves PhD Grant (UI/BD/151312/2021) and C. Amaral contract (DL 57/2016 Norma Transitória; Post-doc grant SFRH/BPD/98304/2013), and for the funds in the scope of the project 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.

OC14 - Identification of the acarological distribution using household dust collected in Coastal regions of Portugal: Aveiro as a case study

Silva, A.[1], Penha, A.M.[2,3], Vieira, A.[1,] Antunes, C.[2,4], Sousa, A.C. [4,6], Pastorinho, M.R.[2,6]
[1]Department of Chemistry, University of Évora, Portugal; [2]Institute of Earth Sciences-ICT, University of Évora, Portugal; [3]Water Laboratory, School of Science and Technology from the University of Évora; [4]Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Portugal; [5]Department of Biology, University of Évora, Portugal; [6]Comprehensive Health Research Centre (CHRC), University of Évora, Portugal,

Email address: mapenha@uevora.pt

Background. The agglomeration of house dust generates the accumulation of a series of potential allergenic agents, including dust mites. These constitute a problem for human health, as they contact individuals through a set of exposure routes, causing allergic diseases. This work aimed to identify which dust mite species were present in house-dust samples in four different houses from Aveiro.

The importance of this study was because there are some species more allergenic than others, which way of controlling is distinct. Thus, by knowing which species are predominant in a humid zone of continental Portugal, it is possible to find effective methods to mitigate the allergies caused, consequently minimizing possible health problems caused by their accumulation. This work is also of great importance once the current acarological map of Portugal, published in 2009, is outdated, mainly due to the climate changes, which, by interfering with the fundamental parameters that manage the life cycle of these species (e.g., humidity, temperature), directly influence the geographical distribution of mite species.

Methods. House dust samples were obtained from vacuum bags and deposits with approximately one month of collection. Samples were sieved (4 meshes) and 4 fractions obtained: 500 – 250 µm; 250 - 125 µm; 125 – 63 µm; and <63 µm. Isolation of specimens was performed by flotation (5M NaCl).

These different fractions were first analysed under a microscope and then by molecular techniques based on genomic analysis, using appropriate *primers* and amplification using RT-PCR (*Real-Time Polymerase Chain Reaction*), followed by agarose gel electrophoresis for RT-PCR confirmation.

Results. Through microscopic analysis it was not possible to identify the individuals in all fractionated samples, given the almost complete loss of their phenotypic characteristics. However, by analysing the results obtained by RT-PCR, it was possible to identify the presence of *Dermatophagoides pteronyssinus*, considered as the dominant specie, in three of the fractions (i.e. 500 – 250 µm; 250 - 125 µm; 125 – 63 µm). The species *D. farinae* was not detected in any of the studied fractions.

Conclusions. The results obtained showed that the analysis of household dust samples with the aim of identifying the species of dust mites present by microscopic observation is not feasible due to the extreme fractionation presented by the individuals, resulting in the almost complete loss of phenotypic characteristics. On the other hand, it is possible to verify that the detection of the species using RT-PCR of the finest fraction obtained (<63 µm) is also not feasible, particularly when there is a small amount of sample. This study allowed us to verify the presence of dust mites in household dust samples, as well as the presence of the species *D. pteronyssinus,* the most common species of dust mites found in household dust. It also allowed determining which of the fractions used were the most appropriate for the identification of the target species by RT-PCR.

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020.

OC15 - Differential effect of ABTS on the laccase and peroxidase-catalyzed degradation of organic pollutants

João Lopes[1,2], Dorinda Marques-da-Silva[1,3], Joaquim Rui Rodrigues[1,3], Paula Videira[2,4], Ricardo Lagoa[1,2]

[1]ESTG-Polytechnic Institute of Leiria, [2]UCIBIO-NOVA University of Lisbon, [3]Associate Laboratory LSRE-LCM-Polytechnic Institute of Leiria, [4]Associate Laboratory i4HB-NOVA University of Lisbon, Portugal

Email address: jrm.lopes@campus.fct.unl.pt

Background. Laccases and peroxidase enzymes can be applied to catalyze the degradation of important organic pollutants, including of polycyclic aromatic carbons (PAHs), dyes and others. Several strategies, from protein engineering to process design, have been investigated to develop efficient and robust environmental technologies based on these enzymes. The use of redox mediators, such as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), is known to improve the catalytic capacities of laccases (laccase-mediator systems), by acting as electron carriers between enzyme and the target compounds. However, the addition of this synthetic mediator to the remediation processes also presents disadvantages and must be optimized. Concerning peroxidases, the effect of ABTS on their ability to oxidize organic pollutants or other molecules has not been studied before.

Methods. Commercially available and commonly used enzymes were adopted for the study: laccase from *Trametes versicolor* and the horseradish peroxidase (HRP). Anthracene and methyl orange were employed as models of environmentally relevant PAHs and organic dyes, respectively. The enzyme-catalyzed oxidation of anthracene, initially at a concentration of 1 mg/L, was determined in degradation assays lasting 24 hours, after which the reaction mixtures were analyzed by HPLC. Methyl orange decolorization was followed in spectrophotometric kinetic assays, starting with initial dye concentrations of 10 mg/L. All assays were carried at pH 5, with laccase 0.1 mg/mL or HRP 0.2 mg/L (100 μM H_2O_2). These concentrations of laccase and HRP corresponded to 85 and 93 mU/mL, respectively, of ABTS oxidizing activity.

Results. Both laccase and HRP without ABTS (enzyme alone) were unable to oxidize anthracene, but could transform the azo dye, although with very different efficiencies. The presence of ABTS in the reaction media assisted the enzyme-catalyzed oxidation of anthracene. Importantly, low micromolar concentrations of ABTS were enough to significantly mediate laccase activity: ABTS 5 μM provided 15±10 and 50 μM 69±4 % degradation of anthracene. Using HRP, at a concentration with similar ABTS oxidizing activity, 50 μM of ABTS provided only 17±7% degradation of anthracene. Methyl orange decolorization by laccase alone was very slow, but addition of ABTS into the reaction media greatly accelerated the dye transformation. ABTS concentrations up to 10 μM could increase one order of magnitude the decolorization rate catalyzed by laccase, in accordance to the results obtained for anthracene. In contrast, HRP transformed methyl orange efficiently and the presence of ABTS showed no beneficial effect.

Conclusions. The present study indicates that low concentrations of ABTS can be used in laccase-mediator systems to balance process efficiency and drawbacks like mediator cost or toxicity. Peroxidase-type enzymes have potential for the degradation of environmental pollutants, but the use of redox mediators requires careful evaluation.

OC16 - Suspension and deposition patterns of microplastics from a 3D printing source

Ana Matias[1], Araújo, M.M.[2], Célia Antunes[3,4], M. Ramiro Pastorinho[3,5]

[1] Department of Biology, University of Évora, Portugal; [2] ARTERIA_LAB, Universidade de Évora; [2]Escola de Artes, Universidade de Évora; [3]Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Portugal; [4] Institute of Earth Sciences-ICT, University of Évora, Portugal; [5]Comprehensive Health Research Centre (CHRC), University of Évora, Portugal

Email address: rpastorinho@uevora.pt

Background. Plastics are ubiquitous, being used in virtually any human activity. Micro (<5 mm) and nanoplastics (<100 nm) result from the degradation of macroplastics or from direct commercial application. Inhalation, dermal contact or ingestion of these hazardous particles can result in risk for humans, with the smaller fractions being translocated to the circulatory and lymphatic systems and being systemically distributed to organs and tissues. The use of 3D printing has been gaining popularity, but 3D printers are a potential source of micro-nanoplastics as they use temperature to model thermoplastic filaments into 3D structures. Very few investigations were performed until now regarding emissions. The objective of the study was to detect the emission and quantify micro-nanoplastic particles (P) and propose mitigation strategies for the printer users.

Methods. Samples were obtained by passive and active methods in order perform calculations of suspended and deposited particles. The passive method consisted of 36 petri dishes (18 glass + 18 polystyrene), at 2 different distances from the printer, that were recovered at a rate of 6/week along 6 weeks. Active samples were obtained using an air sampler (Coriolis) while the printer was working. Samples were processed following a pre-established protocol, colored with Nile Red and observed under a fluorescence microscope. Suspended (/m^3) and deposited (/m^2) particle numbers were calculated.

Results. Microplastics were detected in all samples obtained, both for suspended (13.3±16.4 particles/m^3/hour) and deposited particles (23.9±11.4 particles/m^2/week), both parameters varied but the latter suffered wider variation in absolute numbers. The amount (finer particles more abundant further away: 24.5 vs 5.0 particles/m^2 on average), shape (irregular close to, spherical away from the printer) and size (bigger close to the printer: 100.7 vs 52.7 µm on average) of the particles varied with distance to the printer. Also, the petri dish material influenced these parameters in a nonlinear fashion. Microplastics <10 µm were ubiquitously present in the samples, which lead us to believe that nanoplastics were also present, but were not observed due to the microscope's limit of resolution.

Conclusions. This work showed that 3D printing is a source of microplastics and a potential source of nanoplastics. Moreover, parameters like distance from the printer and the composition of the surface they land into, can influence the characteristics of the available particle, thus creating a very complex pattern of exposure. 3D printers should be located in well ventilated spaces, and workers should vacate the premises during operation hours, since there is direct evidence of inhalable microplastics (<10 µm) and indirect evidence of the emission of highly hazardous nanoplastics (<100 nm).

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020.

S4 - STRUCTURAL BIOLOGY AND MOLECULAR MODELLING / S7 – MEMBRANES AND CELL BIOPHYSICS

S4 - Cellular function is supported by finely orchestrated biomolecular interactions. Macromolecular assemblies coordinate complex biological functions such as transcription, translation, cytoskeleton organization, signalling cascades and secretion pathways. To fully grasp the underlying complexity and dynamics of these molecular machines the combination of integrative Structural Biology and Molecular Modelling approaches is crucial. During this session, experimental and theoretical structural biology studies will be presented revealing the molecular mechanisms of various biomolecules with unprecedented atomic detail.

S7 - Cell membranes are essential to cell function and are crucial for life. They establish an external barrier separating the inner from the outer media and define intracellular compartments (organelles) essential for metabolic processes and molecular trafficking. The coordinated regulation of membrane components (lipids and proteins) has impact in membrane selective permeability and is a feature providing flexibility and robustness to a variety of physiological processes, including cell signalling. Disorders of membrane lipids, dysfunction of membrane proteins and disruption of cell compartmentalization have serious consequences for living cells and organ performance and are related with several diseases. Several of these issues, from the structural to the functional level, will be addressed in this session.

IL7
Ana Luísa Carvalho (UNL)

IL8
M. Rosário Domingues (UAv)

IL9
Maria João Sarmento (CAS, Czeck Republic)

INVITED LECTURES S4/S7

(IL7-IL9)

Ana Luísa Carvalho *Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology; UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology*	A molecular view on the carbohydrate preferences of highly efficient cellulolytic bacteria
Rosário Domingues *CESAM and Mass Spectrometry Center, University of Aveiro*	The use of lipidomics to study membrane phospholipids
Maria João Sarmento *Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Prague, Czech Republic*	The impact of the glycan headgroup on the nanoscopic segregation of gangliosides

Chairs: Cláudio Soares (ITQB NOVA); Graça Soveral (FFUL); Nuno Santos (FMUL)

IL7 - A molecular view on the carbohydrate preferences of highly efficient cellulolytic bactéria

Ribeiro DO[1,2], Costa R[1,2], Viegas A[1,2], Pinheiro B[1,2], Pires VMR[3], Chai W[4], Feizi T[4], Filipe S[1,2], Brás N[5], Fontes CMGA[3,6], Palma AS[1,2,4], Carvalho AL[1,2]

[1]Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal, [2]UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal, [3]CIISA, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal, [4]Glycosciences Laboratory, Faculty of Medicine, Imperial College London, London W12 0NN, UK, [5]LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal, [6]NZYTech Genes & Enzymes, Campus do Lumiar, 1649-038 Lisbon, Portugal

Email address: almc@fct.unl.pt

The plant cell wall is, in its majority, constituted by complex and structurally diverse polysaccharides that are valuable resources for industrial and biotechnological applications. Anaerobic microbial organisms are highly efficient for plant cell wall polysaccharide biodegradation and have evolved a multi-enzyme complex system, the Cellulosome, where catalytic enzymes have appended non-catalytic Carbohydrate Binding Modules (CBMs) that highly potentiate the enzymes' catalytic efficiency. Deciphering at molecular level the mechanisms underlying plant cell wall carbohydrate recognition and deconstruction by different cellulolytic bacteria is crucial to elucidate these complex biological systems, as well as to further promote novel potential applications. The here reported work is focused on a unique approach combining carbohydrate microarrays with X-ray crystallography, to uncover carbohydrate ligands for CBMs and to structurally characterize novel CBM-carbohydrate interactions of two anaerobic bacteria that reside in different ecological niches: *Clostridium thermocellum*, found in soils, and *Ruminococcus flavefaciens* FD-1, present in the rumen of herbivores. Overall, the two bacteria differentially expressed CBM families with different carbohydrate-binding specificities, which may reflect adaptation to substrate availability in their specific ecological niche or the complexity of their Cellulosome. Using the information derived from the high-throughput microarray analysis, and according to their biotechnological relevance or novelty, CBMs and the respective ligands were selected for further biochemical and structural studies, which will be presented.

Acknowledgments This work was supported by Fundação para a Ciência e a Tecnologia (FCT-Fundação para a Ciência e a Tecnologia, I.P.), Portugal, through the project grants PTDC/BBB-BEP/0869/2014 (to ALC), PTDC/BIA-MIB/31730/2017 (to ASP), the fellowships SFRH/BD/100569/2014 (to DR), PD/BD/135517/2018 (to RC) and DL-57/2016 Program Contract (to BP); additionally financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project 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.

IL8 - The use of lipidomics to study membrane phospholipids

Rosário Domingues[1]

[1]CESAM and Mass Spectrometry Center University of Aveiro, 3810-193,Aveiro, Portugal.

Email address: mrd@ua.pt

Background. Phospholipids are main components of cell membranes and also important signaling molecules. Alteration of the lipidome may be associate with alteration in membrane properties, like curvature and fluidity, can cause disfunction of membrane proteins and impair cell signaling, and utmost induce cell malfunction or even cell death.

The cellular and membrane phospholipidome is highly regulated through a set of specific enzymes. Interestingly, membrane phospholipdimoe is quite specific of each type of cell and organelle. The identification of these typical lipidome is still a challenges addressed by modern mass spectrometry based approaches, that aims to address the correlation of lipidome plasticity with health and disease conditions.

Methods. Combined approaches using Chromatography and mass spectrometry techniques have been used to decipher the specific phospholipidome signature of cell membranes. Traditionally thin layer chromatography (TLC) can give information at class level, and more recently liquid chromatography coupled with mass spectrometry (LC-MS) allowed to obtain detailed information and class and lipid molecular specie levels, giving a more broad identification of the phospholipdome of biological samples.

Results. Using lipidomics approaches it was possible to identify the specific lipidome of different type of cell, exemplified here for dendritic cell and cardiomyocytes and also for organelles, like mitochondria. Results illustrate also the deviation of the phospholipidome in disease conditions.

Conclusions. Overall, phopholipidomics can provide new information to unravel membrane properties and funtion, and indication of modified metabolic pathways that can be useful for new therapeutic strategies and to pinpoint new lipid biomarkers of disease.

IL9 - The impact of the glycan headgroup on the nanoscopic segregation of gangliosides

Sarmento MJ[1], Owen MC[2,3], Ricardo JC[1], Chmelová B[1], Davidović D[1], Mikhalyov I[4], Gretskaya N[4], Hof M1, Amaro M[1], Vácha R[2], Šachl R[1]
[1]J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Prague, Czech Republic. [2]CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czech Republic.
[3]Institute of Chemistry, University of Miskolc, Miskolc-Egyetemváros, Hungary.
[4]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Science, Moscow, Russia

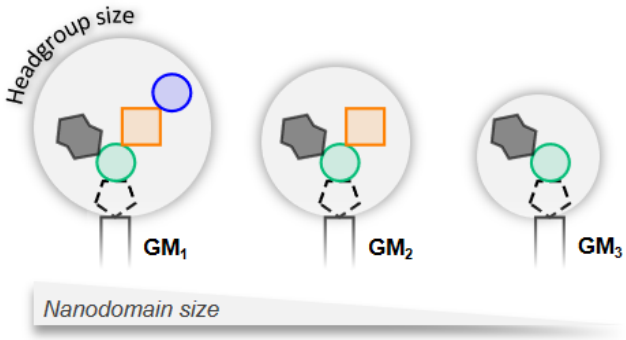
Email address: maria.sarmento@medicina.ulisboa.pt

Background. Gangliosides form an important class of lipids whose large oligosaccharide headgroup and ability to self-organize within lipid membranes results in the formation of nanoscopic platforms. To this day, the molecular reasons for the nanoscopic segregation of gangliosides are not clear.

Methods. To investigate the role of the headgroup in the clustering of gangliosides, we analyzed the ability of gangliosides GM_1, GM_2 and GM_3 to spontaneously self-organize into such nanodomains. To reach nanoscopic resolution and to identify molecular forces that drive the ganglioside's segregation, we combined a novel experimental technique MC-FRET (Förster Resonance Energy Transfer analyzed by Monte-Carlo simulations) with atomistic molecular dynamic (MD) simulations.

Results. We found that these domains range from 7 to 120 nm in radius, are inter-leaflet coupled, and are majorly composed of DOPC and, if present, also by Chol and SM, with the relatively fewer gangliosides. The interactions between gangliosides are dominated by hydrogen bonding network between the headgroups. The corresponding interaction energies decrease by reducing the number of sugar units in the oligosaccharide chain (i.e., from GM_1 to GM_3). The N-acetylgalactosamine sugar moiety of GM_2, however, impairs the stability of GM_2 nanodomains by disrupting hydrogen bonding of neighboring sugars, which is reflected in broad radial distribution functions g(r) for GM_2 and a broad size distribution of GM_2 driven nanodomains. MD simulations suggest the formation of nanodomains to expose the sialic acid by orientating its COO- group facing upwards.

Conclusions. Overall, we provide a comprehensive study that identifies the key factors that drive nanoscopic segregation of gangliosides.



ORAL COMMUNICATIONS S4/S7

OC17	João Alves	Characterization of Cyberlindnera jadinii carboxylate transporters for the improvement of microbial cell factories
OC18	Henrique Fernandes	The Nitrate and Nitrite Reductase Activity of Xanthine Oxidase: a Computational Study
OC19	Paula Alexandra Lopes	Downregulation of aquaporins gene expression by glutamine or cystine-enriched diets in the small intestine of piglets

Chairs: Cláudio Soares (ITQB NOVA); Graça Soveral (FFUL); Nuno Santos (FMUL)

OC17 - Characterization of *Cyberlindnera jadinii* carboxylate transporters for the improvement of microbial cell factories

Maria Sousa-Silva[1,2]*, João Alves[1,2], Daniel Vieira[1,2], Pedro Soares[1,2], Margarida Casal[1,2] & Isabel Soares-Silva[1,2]

[1]Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal
[2]Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Email address: id9021@alunos.uminho.pt

Background. Organic acids display great applicability since these are used as platform chemicals in several industries. The expression of carboxylate transporters in microbial cell factories is crucial for organic acid export and consequently its efficient and cost-effective bioproduction. Herein, we identified and characterized novel carboxylate transporters from the *Cyberlindnera jadinii* yeast.

Methods. The *C. jadinii* transportome was analysed using two approaches, i) identification of *C. jadinii* genes homologous to the carboxylate transporters Jen1 and Ato1, where ten putative transporters were heterologously expressed in the *Saccharomyces cerevisiae* W303-1A *jen1*Δ *ato1*Δ strain for functional characterization, ii) development of a bioinformatics approach where the *C. jadinii NRRL-1542* proteome was downloaded from the NCBI database and explored to determine its predicted transportome. A software tool was designed to retrieve data from an established local database that contains a single representative genome on the species level. Thus, multiple matches within species directly reflect the presence of homologs within the same genome [1]. Genes selected using this tool were expressed in the *S. cerevisiae* IMX1000 strain [2]. In both strategies, transporter activity was evaluated by growth on different carbon sources and measurement of the uptake of radiolabelled carboxylic acids.

Results. In *C. jadinii*, 6 genes homologous to *ScJEN1*, 5 genes homologous to *ScATO1*, and 5 putative carboxylic acid transporters were identified. All proteins were expressed and localized at the plasma membrane. Characterization of newly identified *C. jadinii* transporters is currently underway including phylogenic analysis, transporter specificity, and molecular docking studies.

Conclusions . In this work, we functionally characterized 16 *C. jadinii* carboxylate transporters by heterologous expression in *S. cerevisiae*. These transporters present different specificities for mono, di, and tricarboxylic acids.

References

[1]Ribas D et al. (2018) *FUNGAL GENET BIOL*. 122:1-0
[2]Mans R et al. (2017) *FEMS Yeast Res*, 17:8

OC18 - The Nitrate and Nitrite Reductase Activity of Xanthine Oxidase: a Computational Study

Henrique S. Fernandes[1,2], Sérgio F. Sousa[1,2], José J.G. Moura[3], Luísa B. Maia[3], and Nuno M.F.S.A. Cerqueira[1,2]

[1]Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculdade de Medicina, Universidade do Porto, 4200-319 Porto, Portugal; [2]UCIBIO – Applied Molecular Biosciences Unit, BioSIM - Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, 4200-319 Porto, Portugal; [3]LAQV@REQUIMTE, FCT NOVA, Campus de Caparica, 2829-516 Caparica, Portugal

Email address: hfernandes@med.up.pt

Background. Xanthine Oxidase (XO, EC 1.17.3.2) is a molybdenum-containing enzyme that under hypoxic conditions can catalyze the reduction of NO_3^- to NO_2^- and of NO_2^- to $^{\bullet}NO$. [1] $^{\bullet}NO$ is an important signaling molecule and its deficiency in humans has been associated with some disease conditions such as myocardial infarction, systemic and pulmonary hypertension and gastric ulceration. [2] In this work, the reaction mechanism of XO-catalyzed NO_3^- and NO_2^- reduction to $^{\bullet}NO$ was studied, with an atomic level of detail, using a computational approach.

Methods. The ONIOM QM/MM methodology was employed to characterize all the minima and transition-state structures using the B3LYP/6-31G(d,p):ff99SB scheme with the LanL2dz as pseudo-potential for molybdenum. molUP [3] plugin for VMD was used to prepare all the input files and analyze the results. All the calculations were performed on Gaussian 09.

Results. The results obtained support the $^{\bullet}NO$-forming nitrate and nitrite reductase activities of XO observed experimentally. [1] The reaction mechanism of NO_3^- reduction involves three-sequential steps: NO_3^- binding to the molybdenum ion (at the enzyme active site); heterolytic cleavage of N-O bond; and the enzymatic turnover. In this process, the metal is oxidized from Mo(IV) to Mo(VI), making it ready to start a new catalytic cycle. The reduction of NO_2^- follows the same type of mechanism, but the N-O bond undergoes a homolytic cleavage. Consequently, the metal is oxidized to Mo(V) in the first cycle and then, a new NO_2^- molecule is reduced to $^{\bullet}NO$ leading to oxidation of the metal to Mo(VI), thus closing the enzymatic turnover.

Conclusions. These results go in line with the experimental data regarding the catalytic ability of XO to produce $^{\bullet}NO$ from NO_3^- and NO_2^- reduction. Moreover, the Gibbs free energy profile computed in this work shows that the reduction of nitrate and nitrite are irreversible. These simulations together with the available experimental knowledge about XO provide new insights about the relevance of this enzyme in the $^{\bullet}NO$ production *in vivo*.

References

1. Maia et al. (2018). Redox Biology, 19:274.89.
2. Lundberg et al. (2008). Nat Rev Drug Discov, 7(2):156.167.
3. Fernandes et al. (2018). Journal of Computational Chemistry, 39(19):1344.1353

Acknowledgments: Fundação para a Ciências e Tecnologia (SFRH/BD/115396/2016, 2020.01423.CEECIND, UIDP/04378/2020, UIDB/04378/2020, PTDC/QUI-QFI/31689/ 2017, 01/SAICT/2016 number 022153, CPCA/A00/7140/2020, CPCA/A00/7145/2020).

OC19 - Downregulation of aquaporins gene expression by glutamine or cystine-enriched diets in the small intestine of piglets

Paula A. Lopes[1*], Inês Vieira da Silva[2], Bárbara P. Soares[2], Catarina Pimpão[2], Rui M. A. Pinto[2], José A. M. Prates[1], Graça Soveral[2]

[1]CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Av. da Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal; [2]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal;

Email address: ampalopes@fmv.ulisboa.pt

Background: The regulation of glycerol permeability in the gastrointestinal tract is crucial to control fat deposition, lipolysis and gluconeogenesis. Knowing that the amino acid glutamine is a physiological regulator of gluconeogenesis, whereas cystine promotes adiposity, herein we investigated the effects of dietary supplementation with glutamine and cystine on the transcriptional profile of membrane water and glycerol channels aquaporins (AQPs) in the ileum portion of the small intestine and its impact on intestinal permeability.

Methods: Twenty male piglets with an initial body weight of 8.8 ± 0.89 kg (mean □ SD) were randomly allocated to four dietary treatments with five animals each and received, during a four week-period, a basal diet without supplementation (control) or supplemented with 8 kg/ton of glutamine (Gln), cystine (Cys) or the combination of the two amino acids in equal proportions (Gln + Cys).

Results: No variations were found for piglets' final body weight ($p>0.05$) as well as feed intake ($p>0.05$). Most biochemical parameters (at least $p<0.05$) were found improved in piglets fed the combination of Gln and Cys. The redox status, through the assessment of total antioxidant capacity ($p=0.146$) and glutathione peroxidase enzyme activity ($p=0.838$) was unchanged across dietary treatments. *AQP3* mRNA levels were found predominant over the others (*AQP3* > *AQP1* > *AQP7* > *AQP10* > *AQP9*), regardless the addition of amino acids. A clear pattern of downregulation of *AQP1* ($p<0.001$), *AQP7* ($p=0.001$) and *AQP10* ($p<0.001$) gene expression was found in piglets fed diets enriched in glutamine and cystine, individually or combined, relative to the control without impacting on water ($p>0.05$) or glycerol permeability ($p>0.05$) coefficients. The same trend was verified for *AQP9* mRNA levels, although not reaching statistical significance ($p=0.090$). Conversely, Cys enriched diet upregulated *AQP3* and downregulated glycerol kinase (*GK*), without affecting glycerol permeability ($p>0.05$). The gene expression levels of *mTOR* ($p=0.206$) and *PI3KCG* ($p=0.119$) were kept unchanged by amino acid-enriched diets.

Conclusions: A clear influence on the transcriptional profile of the water channel *AQP1* and aquaglyceroporins *AQP3*, *AQP7* and *AQP10* by glutamine and cystine, individually or combined, was observed, but yet counterbalanced by the lipid bilayer diffusion in what concerns water permeation, and by the interplay of several AQPs isoforms in what concerns glycerol permeation, thus assuring both volume and energetic homeostasis. Due to similarities between pigs and humans, this study hinders the translation of porcine data to humans and warrants further research to validate its findings.

This study was funded by Ajinomoto Animal Nutrition Europe, Indukern Portugal, Lda., and Fundação para a Ciência e a Tecnologia (FCT, Lisbon, Portugal) through projects UIDB/00276/2020 to CIISA, and PTDC/BTM-SAL/28977/2017 and UID/DTP/04138/2019 to iMed.ULisboa. It is also financial supported by FCT Stimulus of Scientific Employment Program to P. A. L. (DL57/2016/CP1438/CT0007).

SYMPOSIUM 5 - SPB-SPN: NEUROBIOLOGY OF AGING AND STRESS

Molecular and physiological changes associated with neurodegenerative diseases are inexorably linked with aging. Age-related gradual decline of organismal homeostasis, which includes changes in ER and oxidative stress, mitochondrial dysfunction, DNA damage, intracellular transport, protein synthesis and turnover, etc., have all been linked with formation of pathognomonic protein aggregates. Thus, advances in our understanding of the molecular basis of neurodegeneration and healthy aging present an imperative and exciting opportunity to identify novel druggable targets and develop more effective interventions.

IL10
Tiago Oliveira (UMin)

IL11
Miguel Castelo Branco (UC)

INVITED LECTURES S5

(IL10-IL11)

Tiago Gil Oliveira
Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho

The impact of chronic stress on the brain lipidome – implications for mood disorders and Alzheimer's disease

Miguel Castelo-Branco
Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), ICNAS; Faculty of Medicine, University of Coimbra

In vivo functional and molecular imaging approaches to study ageing and neurodegenerative disorders

Chairs: Ana Cristina Rego (UC); João Laranjinha (UC);

IL10 - The impact of chronic stress on the brain lipidome – implications for mood disorders and Alzheimer's disease

Tiago Gil Oliveira

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Portugal

Email address: tiago@med.uminho.pt

Chronic stress is a major risk factor for brain disorders, such as depression and Alzheimer's disease, and affects the functioning of the hippocampus, a brain region relevant for learning and memory and emotional response. Since the brain is mainly composed of lipids, and since these molecules are relevant for various key cellular processes, these are likely altered in conditions that lead to brain regional dysfunction. Importantly, recent mass-spectrometry approaches allow the identification of functional and dysfunctional lipidomic signatures, and we have used these strategies to study the effects of chronic stress in the brain. Moreover, we studied the hippocampus at the subregional level along its longitudinal axis, since it was previously shown that the dorsal and ventral poles, in rodents, contribute differentially to stress and emotional responses. These lipidomic signatures allow the identification of candidate lipid signaling pathways and we are currently testing the impact of modulating these pathways with genetic rodent models at biochemical, functional and behavioural levels. Overall, the goal of these projects is to identify lipid pathways relevant for brain function and dysfunction with potential implications for the treatment and diagnosis of brain disorders, such as depression and Alzheimer's disease.

IL11 - In vivo functional and molecular imaging approaches to study ageing and neurodegenerative disorders

Miguel Castelo-Branco

Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), ICNAS;
Faculty of Medicine, University of Coimbra, Portugal

Email address: mcbranco@fmed.uc.pt

Molecular and functional imaging approaches to study brain neurochemistry, structure and function are increasingly being used both in animals and humans to understand healthy ageing and neurodegenerative diseases. Here we discuss our recent research in healthy ageing and neurodegenerative disorders such as Alzheimer's and Parkinson´s disease to show that multimodal research helps provide a unique perspective on disease mechanisms from molecular to systems level. We also provide particular attention to the link between metabolism and neurotransmission, in the context of disease where the link with neurodegeneration remains intriguing, such as diabetes. Finally, we discuss the often overlooked issue of vulnerability towards abnormal ageing in neurodevelopmental disorders.

ORAL COMMUNICATIONS OS1b/S5

OC20	Patrícia Coelho	Impact of SAPAP3 on mitochondrial function in Huntington's disease
OC21	Federico Herrera	N-terminal phosphorylation of Hungtintin exon 1 shifts its liquid-liquid phase separation diagram and alters the kinetics of aggresome formation in mammalian cells
OC22	Ana Branco	Hypoxia-induced quiescence: a new approach to improve UC-MSC therapeutic value
OC23	Pedro Peralta	The role of nonsense-mediated mRNA decay on NKX6-2-associated spastic ataxia 8
OC24	Cândida Dias	Aerobic Glycolysis in the Hippocampus Sustains Extracellular Lactate to Support Neuronal Oxidative Metabolism

Chairs: Ana Cristina Rego (UC); João Laranjinha (UC);

OC20 - Impact of SAPAP3 on mitochondrial function in Huntington's disease

Coelho, Patrícia[1]; Fão, Lígia[1,5]; Nobre, Rui[1,2]; Pereira de Almeida, Luís [1,3]; Peça, João[1,4]; Rego, A. Cristina[1,5]
[1]CNC - Center for Neuroscience and Cell Biology, University of Coimbra; [2]Institute for Interdisciplinary Research, University of Coimbra, (IIIUC); [3]FFUC – Faculty of Pharmacy, University of Coimbra, Coimbra; [4]FCTUC – Faculty of Science and Technology, University of Coimbra, Coimbra; [5]FMUC - Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Email address: patriciardcoelho1212@gmail.com

Background: Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion at the *HTT* gene, also characterized by motor and cognitive impairment and early psychiatric disturbances (e.g. obsessive-compulsive disorder - OCD). Mutant huntingtin (mHTT) affects striatal GABAergic neurons and glutamatergic cortico-striatal synapses and causes, among other hallmarks, mitochondrial dysfunction. Previous studies demonstrated that the postsynaptic scaffold protein SAPAP3, mainly located in striatum, is an important player in OCD, and preliminary data indicate that this protein has several mitochondrial interactors. Therefore, striatal dysfunction linked to early mitochondrial deregulation may involve changes in SAPAP3, and potentially explain HD-related psychiatric disturbances.

Methods: In this work we analyzed SAPAP3 protein and mitochondrial levels in pre-symptomatic (3 m.o.) and symptomatic (6, 10-12 m.o.) YAC128 transgenic (expressing full-length mutant HTT) *versus* WT mice, primary striatal and cortical cultures (13 DIV) from YAC128 *versus* WT mice, and immortalized mouse mutant striatal cells derived from HD knock-in mice (ST*Hdh*$^{Q111/Q111}$) *versus* WT (ST*Hdh*$^{Q7/Q7}$) cells by immunocytochemistry and *western blotting*. Moreover, we studied SAPAP3 involvement on mitochondrial function and dynamics, by both silencing and overexpressing SAPAP3.

Results: Our results showed reduced SAPAP3 total and mitochondrial levels in symptomatic YAC128 mice, mature primary neurons from YAC128 mice and ST*Hdh*$^{Q111/Q111}$ cells, when compared to the respective controls. Interestingly, in YAC128 primary striatal and cortical neurons, SAPAP3 diminished levels were more pronounced at distal neurites, pointing towards a postsynaptic deregulation in HD. Accordingly, colocalization between SAPAP3 and an important scaffold protein, PSD-95, demonstrated decreased puncta number and area, as well as altered SAPAP3 levels. Of relevance, SAPAP3 was shown to be involved in normal mitochondrial function. Silencing of SAPAP3 impaired mitochondrial morphology (e.g. increased roundness), neurite mitochondrial movement and function, and generated higher levels of reactive oxygen species, whereas SAPAP3 overexpression ameliorated all these mitochondrial phenotypes in HD cells.

Conclusions: Our data indicate that SAPAP3 levels control mitochondrial function and that targeting this protein might have a neuroprotective role in HD.

This work was supported by European Huntington Disease Network (EHDN) and by European Regional Development Fund (ERDF), through Centro 2020 Regional Operational Programme: project CENTRO-01-0145-FEDER-000012-HealthyAging2020, the COMPETE 2020-Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, I.P.: project POCI-01-0145-FEDER-007440, and UIDB/04539/2020.

OC21 - N-terminal phosphorylation of Hungtintin exon 1 shifts its liquid-liquid phase separation diagram and alters the kinetics of aggresome formation in mammalian cells

Quezada A.[1,2,3], Kovalchuk D.[1], Herrera F[1,2,3]

[1]Cell Structure and Dynamics Laboratory, Faculty of Sciences, University of Lisbon, 1749-016, Lisbon, Portugal, [2]BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016, Lisbon, Portugal, [3]MOSTMICRO Research Unit, Instituto de Tecnologia Química e Biológica (ITQB-NOVA), Universidade Nova de Lisboa, Oeiras, Portugal.

E-mail: fherrera@fc.ul.pt

Background. An extended polyglutamine (polyQ) tract >35 glutamines in the first exon of huntingtin (HttEx1) is the cause of Huntington disease (HD), which major histopathological feature is the presence of huntingtin inclusions in the striatum. HttEx1 is an intrinsically disordered region with a strong tendency to aggregate. In vitro, in the absence of the cellular machinery, the classic amyloidogenesis view has prevailed to describe the process of Httex1 aggregation. However, in the crowded and dynamic intracellular environment, HttEx1 forms an aggresome in the perinuclear region of cells and liquid-like condensates by liquid-liquid phase separation (LLPS), as a previous step to the formation of amyloid-like fibrils. In this study, we investigate the effect of the polyQ tract length and the phosphorylation of HttEx1 on the kinetics of aggresome formation and liquid-liquid phase separation.

Methods. We used Biofluorescence Complementation (BiFC) assays to image the formation of aggresome and liquid condensates of HttEx1 in HeLa cells. We measured the kinetics of aggresome formation and produced phase diagrams using time-lapse quantitative fluorescence microscopy in live cells in combination with Super-resolution radial fluctuations. The interplay between aggresome formation and the cytoskeleton of cells was further characterized in both live and fixed cells.

Results. A single mutation mimicking phosphorylation on serine 13 (S13D) confers the condensates with a different phase separation regime that resembles a spinodal decomposition. This phenomenon is independent of the polyQ length since a wild type variant also shows this behavior. On the other hand, the PolyQ length has a great effect on the kinetics of aggresome formation. Wild-type HttEx1 do not form aggresomes, and a variant with 47Q shows slower kinetics of formation compared with a variant containing 97Q (97QHttEx1). Phosphomutant variants show a less variability on the kinetics of aggresome formation compared with a non-mutant form of 97QHttEx1, indicating that electrostatic interactions on the N17 region are playing a relevant role on this cellular phenomenon.

Conclusions. We found that the length of the polyQ tract has a significant influence on aggresome formation, a phenomenon that is driven by the quality control system of the cell. In contrast, phosphorylation on the N17 region of HttEx1 has a greater effect on liquid-liquid phase separation and dynamics of the condensates. Our results show that different structural factors of the protein can drive the diverse processes of Httex1 aggregation in live cells.

OC22 - Hypoxia-induced quiescence: a new approach to improve UC-MSC therapeutic value

Branco A [1,2], Moniz I [1,2], Almeida-Santos T [2,3], Ramalho-Santos J [1,4]

[1]Biology of Reproduction and Stem Cells Group, Center for Neuroscience and Cell Biology, Portugal, [2]Faculty of Medicine, University of Coimbra, Portugal, [3]Reproductive Medicine Unit, CHUC, Portugal, [4]Faculty of Sciences and Technology, University of Coimbra, Portugal.

Email address: abranco@cnc.uc.pt

Umbilical cord mesenchymal stem cells (UC-MSCs) exhibit great therapeutic potential due to their homing ability, differentiation capacity and immunomodulatory properties. Nevertheless, the success of UC-MSC based therapies is still limited by the *in vitro* expansion outside their natural physiological environment (ranging from 1-5% of O_2). Previous reports on MSCs state that moderate hypoxia increases function and proliferation rate, yet in this study we hypothesize that UC-MSCs exposed to specific O_2 levels can also achieve cellular quiescence that, by minimizing cell activity and energetic demand, improves cell survival, stemness and therapeutic function.

UC-MSCs were cultured under <1%, 5% and 21% O_2 and treated with different concentrations of the hypoxia mimicking agent Cobalt Chloride ($CoCl_2$). While cell viability was not affected by hypoxia, experimental conditions generate different effects: as expected, UC-MSCs cultured under 5% O_2 displayed higher population doubling than cells cultured in 21% O_2. Contrary to what was formerly described, however, culture under <1% or with 250μM $CoCl_2$ led to a significant decrease in proliferation, comparable to that of MSCs treated with INK-128, a known mTOR inhibitor. Concomitantly, protein analysis showed that the mTOR effectors 4EBP1 and S6K1 were particularly downregulated in cells exposed to severe hypoxia suggesting a decrease in protein biogenesis. In contrast, phosphorylation of the mTOR target Akt was upregulated in MSCs cultured in <1% O_2 compared to 5% O_2, suggesting a decrase in cell vulnerability. In addition to HIF-1a, AMPK, a known mTOR inhibitor, was upregulated in every hypoxic condition, including $CoCl_2$, suggesting that this compound might affect MSCs and mTOR in a HIF-independent manner. A switch to anaerobiotic glycolysis and inhibition of mitochondrial respiration is implied by the rise of LDHA phosphorylation in each condition, yet downregulation of COX IV was only observed in cell lines treated under severe hypoxia and $CoCl_2$. Regarding stemness, <1% O_2 caused an increase in the osteogenic marker osteocalcin in MEM medium, suggesting that near anoxia might favor UC-MSC differentiation into osteogenic cell lines.

In this study, we expect to provide valuable information on the effects of $CoCl_2$ and different hypoxic settings on the therapeutic value of UC-MSCs.

This work was funded by the Fundação para a Ciência e a Tecnologia under the project STEM@REST (CENTRO-01-0145-FEDER-028871).

OC23 - Protein complementation on NKX6-2 truncated proteins associated with spastic ataxia 8

Ferreira-Peralta P.[1,2], Letra-Vilela R.[1,2,3], Gama-Carvalho M.[2], Romão L.[4], Herrera F.[1,2,3]

[1]Cell Structure and Dynamics Laboratory, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal, [2]BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal; [3]MOSTMICRO Research Unit, Instituto de Tecnologia Química e Biológica (ITQB-NOVA), Universidade Nova de Lisboa, Oeiras, Portugal; [4]Centro de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, 1649-016 Lisboa, Portugal.

Email address: PedroPeralta@fc.ul.pt

Background. NKX6-2 is a transcriptional repressor involved in the regulation of various genes associated with cell fate determination of neurons, oligodendrocytes, or pancreatic beta cells, as well as glucagon production, among other phenomena. Loss-of-function mutations in NKX6-2 are the cause of Spastic Ataxia 8 (SPAX8), a rare recessive hereditary disease, that leads to an early onset hypomyelinating leukodystrophy due to the lack of oligodendrocyte maturation. The most common disease-associated NKX6-2 mutations involve the formation of premature termination codons (PTCs). This study focuses on 5 SPAX8-associated PTCs that are the result of single nucleotide substitutions or frameshifts. These PTCs will render the nascent mRNA a potential target for degradation via non-mediated mRNA decay (NMD). The small fraction of mRNA that survives NMD and is eventually translated to protein will produce truncated, non-functional NKX6-2 proteins. We aim to overcome these two issues to lay the groundwork for the development of new therapies for SPAX8.

Methods. Using site-directed mutagenesis, we were able to recreate SPAX8-related mutations on a NKX6-2+Venus fusion protein. We inserted the DNA response element for the NKX6-2 protein in a pRL miniTK-luciferase system, enabling an easy measurement of NKX6-2 activity as a transcriptional repressor. A complementary NKX6-2+Flagtag+P2A+T2A+mCherry fusion protein was used for protein complementation assay. The effect was verified by microscopy assay, western blot, and in the future luciferase activity assay.

Results. SPAX8-causing mutations in NKX6-2 lead to an even distribution around the cell, signaling the loss of the nuclear localization signal, and in some cases protein aggregation. Constitutive expression of NKX6-2 brings about a conformation change in the cells. Protein complementation shows an increase of truncated NKX6-2 in the nucleus, however, there wasn't a full recovery of protein translocation to the nucleus. Soon, we will begin the luciferase activity assay, to verify the recovery effect of protein complementation.

Conclusions. We have confirmed some of the problems caused by SPAX8-causing mutations in NKX6-2 at the cellular level while bringing the possibility of using protein complementation to solve this issue. This study progresses towards the verification of function recovery, as well as, the effect of NMD on the SPAX8-causing mutations. While our approach is currently restricted to NKX6-2/SPAX8, this proof-of-concept could be applied to other genetic disorders involving PTCs, which are associated to one-third of inherited human diseases.

OC24 - Aerobic Glycolysis in the Hippocampus Sustains Extracellular Lactate to Support Neuronal Oxidative Metabolism

Dias, C.[1,2], Fernandes, E.[1], Barbosa, R. M.[1,2], Laranjinha, J.[1,2], Ledo, A.[1,2]

[1]Faculty of Pharmacy, University of Coimbra, Portugal, [2]Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Email address: candida.dias@cnc.uc.pt

Background. The concept of neurometabolic coupling is tightly bond to that of aerobic glycolysis. Robust evidence suggests that neural cells display complimentary energy metabolism profiles: while neurons are markedly oxidative, astrocytes appear to rely more on the glycolytic pathway. Here, pyruvate is converted to lactate even when O_2 is readily available – aerobic glycolysis – and the latter is readily used by neurons to fuel oxidative phosphorylation. This is the basis of the Astrocyte-to-Neuron Lactate Shuttle. Herein we investigated the interplay between glucose and lactate usage in supporting oxidative metabolism in hippocampal slices, a model that preserves the functional interaction between neurons and glia.

Methods. A multimodal approach was used, combining high resolution respirometry and microbiosensor technology to study the role of lactate as a metabolic substrate in the hippocampus on a dynamic and quantitative basis. The slice oxidative metabolism was evaluated through high resolution respirometry, measuring whole slice oxygen consumption rates (O_2 flux). Additionally, extracellular lactate dynamics were evaluated by amperometry using carbon fiber microelectrode-based lactate biosensors. These were evaluated under resting and stimulated conditions and by changing the composition of the slice bathing medium in glucose and lactate content.

Results. We found that tissue basal O_2 flux increased as a function of the glucose concentration (0.1-15 mM), reaching a maximum at 10 mM. Furthermore, addition of exogenous lactate further increased O_2 flux by ~9% while inhibition of neuronal lactate uptake decreased O_2 flux, indicating that neurons uptake and metabolize lactate under non-stimulated conditions. Measurement of extracellular lactate in hippocampal slices using amperometric biosensors revealed lactate is continuously produced from glucose and released into the extracellular space at rest, with an average $[lactate]_{ECS}$ of 0.37 ± 0.06 mM observed for glucose concentrations above 5 mM. Stimulation of hippocampal slices with KCl (60 mM) led to a significant increase in O_2 flux (~200%) and a transient decrease in extracellular [lactate] (355 µM), indicative of an increase in net lactate consumption by the tissue. Both measured responses were significantly decreased by inhibition of lactate uptake by neurons, corroborating the hypothesis that, when stimulated, neurons uptake extracellular lactate to support oxidative metabolism.

Conclusions. This work supports the notion that glucose is continuously converted to lactate, which is released into the extracellular space in hippocampal tissue and that neurons uptake this lactate as an oxidative fuel under both resting and stimulated conditions.

Acknowledgments. This work was financed by the European Regional Development Fund (ERDF), through the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, under projects POCI-01-0145-FEDER-029099, POCI-01-0145-FEDER-028261, and UIDB/04539/2020. CD acknowledges PD/BD/114371/2016.

SYMPOSIUM 6 - FUNCTIONAL GENOMICS AND SYSTEMS BIOLOGY

The structure, regulation and integrity of genome are essential for cell division, organims´ development and adaptation to environmental stimuli. With Biology entering the digital era, we are immersed in a wealth of information originating in high-throughput experiments. These data enable us for the first time to study life at the level unimaginable before and understand the entire living systems from molecules to cells, from organisms and communities to ecosystems.
This session will outline the great diversity in the epigenomics field, DNA regulation and remodeling, DNA and RNA methylation, new ways of post-translational modifications, by presenting data from diverse model systems clinical research and new insights in clinical research.

IL12
Cecília Arraiano (UNL)

IL13
Carmen Jerónimo (IPO-Porto; ICBAS-UP)

INVITED LECTURES S6

(IL12-IL13)

Cecília Arraiano
Instituto de Tecnologia Química e Biológica António Xavier (ITQB) Molecular, Structural and Cellular Microbiology (MOSTMICRO)

The Expanding Universe of RNAs

Carmen Jerónimo
Department of Pathology; Portuguese Oncology Institute of Porto (IPO Porto) & Department of Pathology and Molecular Genetics Abel Salazar Biomedical Sciences Institute (ICBAS)

DNA and RNA methylation in urological cancer: from biology to clinical biomarkers

Chairs: Cecília Arraiano (UNL); Sandra Viegas (ITQB-UNL)

IL12 - The Expanding Universe of RNAs

Cecília Maria Arraiano

Instituto de Tecnologia Química e Biológica António Xavier,
Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

cecilia@itqb.unl.pt

Biological processes can not be fully understood without a deep understanding of RNA metabolism. In 2006 and 2009 Nobel prizes were dedicated to research in the field of RNA and more recently the power of small RNAs and CRISPr technology has given a new perspective to Molecular Biology and led to another Nobel in 2020. Recently, RNA based vaccines against SARS-CoV2 RNA virus, have been successful in holding the COVID-19 pandemic crisis, and this brought new interest on RNA in the media.

Our laboratory has been focused in the study of RNA degradation mechanisms and the characterization of enzymes and RNA chaperones that mediate RNA decay. Namely we have studied RNase II family of ribonucleases in the maturation, degradation, and quality control of mRNAs and functional non-coding small RNAs, and we have extended our research to eukaryotes to further understand the role of RNases in global regulation and Disease. Our studies have been also applied to areas of Biotechnological interest and Health, and we have been involved in European Projects on Synthetic Biology to reprogram bacteria for biotechnology use. Recently we have characterized the mechanism of action of the two SARS-CoV2 ribonucleases which have shown to be prominent targets for the development of novel antiviral drugs.

A plethora of new RNAs is emerging every month, RNAs are shown to be Important for Intra and Inter species communication, novel roles are being attributed to functional RNAs, and more surprises are yet to come…

The intent of this talk will be to refresh your knowledge on RNA and to encourage you to learn more about the **The Expanding Universe of RNAs**!

IL13 - DNA and RNA methylation in urological cancer: from biology to clinical biomarkers

Carmen Jerónimo, Ph.D.

Head of Cancer Biology & Epigenetics Group - Research Center & Biobankof the Department of Pathology; Portuguese Oncology Institute of Porto (IPO Porto) & Invited Full Professor at the Department of Pathology and Molecular Genetics Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Portugal

Epigenetic mechanisms are well established drivers of malignant transformation. Among these, aberrant DNA methylation is, by far, the most widely studied. Importantly, these DNA modifications and proteins that recognize them gave rise to novel cancer biomarkers, intended for early detection and diagnosis, assessment of prognosis, and prediction of response to therapy. Importantly, the first FDA approved drugs that exploit the gained knowledge has been widely used to combat some cancers. In addition to those DNA modifications, nucleotide modifications in RNA (epitranscriptomics) can change the coding of messenger (mRNAs) and noncoding (ncRNAs) RNAs, being recently implicated in several pathologies including cancer. Moreover, similar to the findings of epigenetics in DNA, groups of proteins have been identified that specifically recognize and bind modified nucleotides thereby affecting RNA's fate, and cellular differentiation and, ultimately tumorigenesis.

In my talk, I will focus on the major recent findings obtained by our research team regarding DNA and RNA epigenetic mechanisms implicated in urological cancers, as well as the potential usefulness as biomarkers for detection and prognostication in these malignancies.

SYMPOSIUM 8 - SBBQ - PROTEINS IN HEALTH AND ENVIRONMENT

Virtually all the important cellular processes such as cell division, cell motility, organelle remodelling and intracellular trafficking depend on the precise localization of proteins and the dynamics of their interactions. Protein folding and trafficking pathways in prokaryotes and eukaryotes are among the most fundamental processes in all of biology. Understanding the mechanisms of protein folding and misfolding regulation and protein trafficking is crucial to understand the role of proteostasis in physiological and pathological conditions, ranging from metabolic imbalance and aging, neurodegeneration, infections, immune disorders, tumorigenesis and cancer therapy. This session will cover the topics of protein localization, structure and dynamics, by different approaches from biophysics to cell biology, including their impact on human disease focusing on their impact on human diseases and contribution to diminish the ecological footprint of human activities.

IL14
Ricardo Louro (ITQB-UNL)

IL15
Eduardo Sousa (Federal University of Ceará, Brasil)

INVITED LECTURES S8

(IL14-IL15)

Ricardo O. Louro
Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova Lisboa

Metalloproteins fighting infection and climate change: Iron-cold iron, is master of them all

Eduardo H. S. Sousa
Universidade Federal do Ceará, Brazil

Oxygen heme-based sensors in nature and medicine

Chairs: Miguel Castanho (UL); José Moura (UNL)

IL14 - Metalloproteins fighting infection and climate change: Iron-cold iron, is master of them all

Ricardo O. Louro

ITQB-NOVA, Oerias, Portugal

Email: louro@itqb.unl.pt

Background. Microbe-mineral interactions underpin numerous biogeochemical cycles of the elements that are essential for sustaining life in the vast majority of ecological niches. Photoferrotrophism and anaerobic respiration of metallic minerals (e.g., iron oxides) are two of the most ancient metabolisms still present on Earth. Photoferrotrophism has been singled out as a key metabolism for the deposition of Banded Iron Formations that predate the Great Oxidation Event, since in this metabolism, bacteria use ferrous iron as electron source for photosynthesis. Microorganisms performing both kinds of metabolism, collecting electrons from solids or delivering them to solids can colonize bioelectrochemical devices. In these, microorganisms growing on the anode can generate electrical current using carbon sources as cheap as wastewater, whereas microorganisms growing on the cathode can synthesize compounds of industrial interest by capturing CO_2 and collecting the current from the electrical circuit. These systems enable simultaneous wastewater treatment with the production of valuable commodities but using a process that is CO_2 negative, contributing to averting catastrophic climate change. Both photoferrotrosphim and anaerobic respiration of metallic minerals requires numerous iron proteins in particular multiheme cytochromes and iron-sulfur proteins. Given that iron is insoluble in aerobic conditions this is a limiting factor for microbial growth in environments as diverse as the open ocean or within eukaryotic hosts of pathogens. This heavy burden on iron scavenging from the environment is overcome using siderophores, small molecules that have some of the highest affinities for ferric iron, allowing its solubilization and cellular uptake.

Stimulation of productivity of the open ocean with the concomitant capture of atmospheric CO_2 is an endeavor of sufficient magnitude to impact the current trajectory of global warming. Also, interfering with the iron-scavenging strategies of pathogens provides a strategy for infection control. Thus, at this point of iron scavenging and capture lies a nexus where research can have an impact both in averting catastrophic climate change and in manipulating host-pathogen interactions.

Methods: A combination of methods was involved in the exploration of these metabolic processes. NMR spectroscopy for the structural and functional characterization of (metallo)proteins and their interactions, X-ray crystallography for structural characterization of the proteins, bioelectrochemistry and stopped-flow for the study of redox reactions.

Results. We achieved the structural and functional characterization of multiheme cytochromes and HIPIPs involved in photoferrotrophism and mineral respiration and identified their roles in these processes. We also determined the structural and functional characterization of key iron scavenging (metallo)-proteins from oceanic organisms and from opportunistic pathogens and their physiological impact.

Conclusions. The characterization of the pathways, used by microorganisms for exchanging electrons with extracellular solids revealed targets for domesticating these microorganisms to achieve optimal performance in bioelectrochemical technologies. The structural and functional characterization of iron scavenging proteins opens the opportunity of a rational design of strategies that interfere with their activity and tune the microbial access to iron in biotechnological applications or in infection control.

IL15 - Oxygen heme-based sensors in nature and medicine

Eduardo H. S. Sousa

Universidade Federal do Ceará, Brazil

Heme-based sensor are found in all kingdom of nature, involved in a myriad of physiological processes such as symbiosis, RNA degradation, vasodilation, biofilm regulation, virulence, dormancy, among others. These usual multi-domain proteins sense and respond to diatomic gaseous molecules (e.g., O_2, CO, NO). Many oxygen sensors have been discovered and their molecular mechanism of functioning have been unfolded, which has illustrated their diversity. FixL is a prototype of an oxygen heme-based sensor involved in the regulation of symbiosis in Rhizobia, which has helped to a better understanding of this family of proteins. Indeed, this protein sensor shares some similarities to DevS (DosS) and DosT, two other oxygen sensors found in *Mycobacterium tuberculosis* (Mtb). These latter sensors are involved in the process of Mtb dormancy. During the last decade, a series of studies carried out by our lab and others have unfolded how these Mtb proteins work, making clearer that an even more complex network of signaling may be involved. The Mtb dormancy process is very important and has been associated to the lengthy treatment of tuberculosis (at least 6 months), including the lack of expectation for eradication of this disease. By understanding these systems along with other associated processes (e.g. nitrate/nitrite fate in dormant Mtb), there is a potential to development new anti-TB therapy as well. This talk is going to describe some advances on oxygen heme-based sensors, mainly on DevS, and current perspectives.

References

1. Lopes et al., Coord. Chem. Rev. 445 (2021) 214096
2. Sousa et al., Coord. Chem. Rev. 423 (2020) 213476
3. Lopes et al., Dalton Trans., 2020, 49, 15988

ORAL COMMUNICATIONS S8

OC25	Anaísa Coelho	One way or another: What lies behind cytochromes belonging to the NapC/NirT family with opposite electron transfer directionality?
OC26	Margarida Saramago	Targeting SARS-CoV-2 ribonucleases to combat COVID-19
OC27	Joana Rita Sousa	Proteomic profiling of Streptomyces aculeolatus: protein markers involved in the biosynthesis of bioactive metabolites
OC28	Ana Cláudia Leite	Cell cycle-dependent regulation of ATP synthase beta subunit
OC29	Laura Carreira	Changes in salivary proteome in patients with dry mouth syndrome- the effect of pilocarpine therapy
OC30	Ricardo Soares	Exploration of the Desulfuromonadales “cytochromome”. Purification of multiheme cytochromes involved in the extracellular respiration of Desulfuromonas acetoxidans

Chairs: Miguel Castanho (UL); José Moura (UNL)

OC25 - One way or another: What lies behind cytochromes belonging to the NapC/NirT family with opposite electron transfer directionality?

Anaísa Coelho[1], Abhiney Jain[2], Joana Madjarov[1], Ricardo O. Louro[1], Jeffrey A. Gralnick[2], Catarina M. Paquete[1]

[1]Inorganic Biochemistry and NMR laboratory, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal, [2]BioTechnology Institute and Department of Plant and Microbial Biology, University of Minnesota, USA

Email address: anaisancoelho@itqb.unl.pt

Background. Electroactive microorganisms have attracted significant interest for the development of novel biotechnological systems for the sustainable production of energy and added-value products. These organisms perform extracellular electron transfer which has been demonstrated to be performed by numerous multiheme *c*-type cytochromes. One of these proteins is the membrane-bound tetraheme cytochrome *c*, CymA, a member of the NapC/NirT family of quinol dehydrogenases. It has been proposed that in *Shewanella oneidensis* MR-1 CymA acts as a hub for electron transfer to multiple periplasmic partners, namely STC and FccA, functioning as a quinol oxidase during Fe(III) reduction. Interestingly, the freshwater chemolithoautotroph *Sideroxydans lithotrophicus* ES-1 also contains a NapC/NirT family tetraheme cyctochrome containing a protein encoded by Slit_2495 that has been proposed to function as the quinone reductase during Fe(II) oxidation based on its sequence similarity with CymA. Therefore, the characterization of these two proteins belonging to the NapC/NirT family regarding its biochemical and electrochemical properties is of great interest to obtain insights regarding the physiological function of these molecules, and understand their mode of action.

Methods. Slit_2495 was produced by recombinant expression in *Escherichia coli* using the accessory plasmid pEC86, purified using affinity chromatography and characterized biochemically by UV-visible spectroscopy, Mass-spectrometry, Resonance Raman spectroscopy, Cyclic Voltammetry, and NMR spectroscopy. The bioelectrochemical experiments were performed in anaerobic conditions and the bacterial growth profile were monitored for approximately 40 h in simple bioelectrochemical reactors with a three-electrode setup. NMR spectroscopy was used to investigate the interaction between Slit_2495 and STC and FccA from *S. oneidensis* MR-1.

Results. The ^{1}H-1D-NMR spectrum of Slit_2495 in the oxidized state exhibits the typical features of a cytochrome with low-spin hemes that are axially coordinated by strong-field ligands. Cyclic voltammetry showed that CymA and Slit_2495 have similar redox properties. The differences detected in the growth of the *S. oneidensis* strains carrying Slit_2495 and CymA are almost insignificant indicating that Slit_2495 can replace CymA in *S. oneidensis* MR-1. By NMR spectroscopy it is possible to observe an interaction between Slit_2495 and STC and FccA from *S. oneidensis* MR-1.

Conclusions. Our preliminary data have revealed differences in the redox behavior and in the NMR spectra of CymA and Slit_2495. Our bioelectrochemical experiments revealed that Slit_2495 can replace CymA, demonstrating that Slit_2495 functions as a quinol oxidase in *S. oneidensis* MR-1. This information is the first step in the complete elucidation of the mode of action of Slit_2495, a knowledge that is still lacking and is crucial to use and improve this organism towards practical biotechnological applications.

OC26 - Targeting SARS-CoV-2 ribonucleases to combat COVID-19

Saramago M, Bárria C, Costa V, Souza CS, Viegas SC, Domingues S, Lousa D, Soares CM, Arraiano CM, Matos RG

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal

Email address: margaridasaramago@itqb.unl.pt

Background. We are facing a global pandemic triggered by SARS-CoV-2, with devastating consequences for healthcare and social-economic systems. The understanding of fundamental aspects of this virus is of extreme importance. We have focused our attention on the RNase nsp14, a multifunctional protein that harbors two distinct activities, an N-terminal 3'-to-5' exoribonuclease (ExoN) and a C-terminal N7-methyltransferase (N7-MTase), both with critical roles in Coronavirus (CoV) life cycle. The ExoN activity is crucial for the proofreading activity during viral replication, while the N7-MTtase activity is essential for the formation of a functional 5' RNA cap structure, critical for stability and translation of CoV mRNAs. ExoN knockout mutants of SARS-CoV-2 are non-viable, showing up as a prominent target for the development of antiviral drugs. Nsp14 ExoN activity is stimulated through the interaction with the nsp10 protein, whose role is also reinforced by its pleiotropic function during viral replication. Nsp10 acts as a cofactor that also boosts nsp16 2'-O-MTase activity, constituting a key regulator of viral RNA synthesis and degradation. Due to its central role, nsp10 is also a promising target for anti-coronavirus therapy.

Methods. In this work, we have purified both nsp10 and nsp14 proteins, and performed the first biochemical characterization of the complex nsp14-nsp10 from SARS-CoV-2. We have also modelled this complex based on the 3D structure of the complex from SARS-CoV (PDB ID 5C8S).

Results. Here, we confirm the 3'-5' exoribonuclease and MTase activities of SARS-CoV-2 nsp14, and the critical role of nsp10 in upregulating the ExoN activity *in vitro*. Mutation of conserved residues on the nsp10 surface, highlighted on our 3D model of the nsp14-nsp10 complex, are shown to have a strong impact on ExoN activity. We have managed to map key nsp10 residues involved in its interaction with nsp14, all of which are also shown to be essential for stimulation of the ExoN activity by nsp10. This reinforces the idea that a stable interaction between nsp10 and nsp14 is strictly required for the nsp14-mediated ExoN activity of SARS-CoV-2.

We have also studied the role of conserved DEDD catalytic residues of SARS-CoV-2 nsp14 ExoN. Our results show that motif I of ExoN domain is essential for the nsp14 function revealing some striking differences in the functionality of nsp14 on SARS-CoV-2, which can have implications to viral pathogenesis.

Conclusions. The nsp10-nsp14 interface is a recognized attractive target for antivirals against SARS-CoV-2 and also other Coronaviruses. Additionally, some nsp10 residues can be targeted to inhibit also nsp16-nsp10 complex formation, leading simultaneously to inhibition of nsp14 ExoN and nsp16 2'-O-MTase activities. This work is a basis for discovering inhibitors targeting the specific amino acids here reported in order to disrupt the assembly of this(ese) complexe(s), potentially interfering with CoVs replication.

OC27 - Proteomic profiling *of Streptomyces aculeolatus*: protein markers involved in the biosynthesis of bioactive metabolites

Sousa J. R.[1], Fernandes A.[1], Costa L.[1], Gaudêncio S.P.[1], Almeida M.G.[1,2]
[1]UCIBIO - Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Portugal., [2]CiiEM - Instituto Universitário Egas Moniz, Campus Universitário, Portugal.

Email address: jrl.sousa@campus.fct.unl.pt

Background. Actinomycetes *Streptomyces aculeolatus* belong to the MAR4 streptomycete lineage and are known for producing secondary metabolites, mainly hybrid isoprenoids (HI) [1]. These compounds have great potential as antibacterial, antifungal, and anticancer agents, which makes them attractive to the pharmaceutical industry [1,2]. In previous works, a total of 400 actinomycetes were isolated from marine sediments collected from the Madeira Archipelago, six of which belong to the *S. aculeolatus* species, as determined by a phylogenetic analysis based on the sequencing of the 16S rRNA gene [3]. These strains were able to produce HI with distinct bioactivities. In this context, we aim at characterizing the proteins involved in the synthesis of the main bioactive compounds generated by the strains PTM-29, PTM-129, PTM-346 and PTM-398. To achieve this goal, we performed a differential proteomic analysis based on two-dimensional electrophoresis (2DE).

Methods. *S. aculeolatus* cells from three individual growths were isolated and purified from marine sediments samples and grown in a starch, yeast extract, peptone and seawater based medium. Cells were harvested and lysed in a French-Press, followed by a centrifugation to eliminate the cell debris. The total protein extract was analyzed by 2DE. The 1st dimension (isoelectric focusing) was performed in IPG strips (11 cm) with a 4-7 pH gradient, using the Ettan IPGphor 3 system. The 2nd dimension (SDS-PAGE) was carried out in 12,5% polyacrylamide gels, which were stained with colloidal Coomassie Blue. The statistical image analysis and quantification of relative protein abundances (ANOVA p-value 0.05) was performed using the Melanie 7.0.6 software.

Results. A preliminary analysis of the 2D gels enabled the identification of 691, 694, 740 and 866 protein spots for the strains PTM-29, PTM-129, PTM-346 and PTM-398 respectively. The overall proteome of strains PTM-29, PTM-129 and PTM-398 was similar, revealing numerous proteins with identical pI and high molecular weight. Interestingly, the 2D gels of strain 346 showed many differences in comparison with those from the three other strains, indicating the presence of new proteins, aside a differential expression of common peptides. Upon gel matching (two-by-two) and statistical validation of the relative abundance of protein spots in the gel (> 2 fold), we found 82 and 113 spots differentially expressed between the PTM-29 and PTM-129 gels and between the PTM-346 and PTM-398 gels, respectively.

Conclusions. The preliminary analysis of the *S. aculeolatus* (strains PTM-29, PTM-129, PTM-346, PTM-398) 2D gels suggests that there are important differences in the protein profiles obtained in the same growth conditions. Noteworthy, the proteome of strain PTM-129, considered as a control (i.e. no biological activity), was more similar to those from other strains, than the proteome of strain PTM-346, which shows several biological activities. Unidentified differences at the genome level, and distinct biosynthetic efficiencies might be responsible for such diversity. The complete analysis of the gels is underway. Next, we intend to identify the differentially expressed proteins by mass spectrometry (MS) techniques.

References

[1] Gallagher KA, Jensen PR. BMC Genomics, 2015;16 (960): 1-13; [2] Bin Cheng Y, *et al*. W. European J. Org. Chem., 2013: 3751–57. [3] Prieto-Davó A, et al. Front Microbiol, 2016; 7: 1-12.

OC28 - Cell cycle-dependent regulation of ATP synthase beta subunit

Leite A.C.[1,2,3], Martins T.S.[1,2,3], Costa V.[1,2,3], Pereira C.[1,2]

[1]i3S Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [2]IBMC Instituto de Biologia Celular e Molecular, Universidade do Porto, Porto, Portugal; [3]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Email address: ana.leite@i3s.up.pt

Background. Mitochondrial ATP synthase is an enzyme complex that generates ATP and is a key determinant of mitochondrial function. Previously, we showed that the 2Alike protein phosphatase Sit4 promotes the dephosphorylation of ATP synthase catalytic beta subunit (Atp2 in yeast) at Thr124/317. Phosphorylation at Thr124/317 upregulates Atp2 levels, leading to an increase in ATP synthase activity and ATP production [1]. Using *in silico* analysis, four kinases were predicted as potential Atp2 regulators, namely Pkc1, Ipl1, Cdc5 and Hrr25. Likewise the phosphatase Sit4, these four kinases play prominent roles in cell cycle regulation suggesting the idea that Atp2 phosphoregulation may be cell cycle related. Here, we set out to investigate if Atp2 phosphorylation is cell cycle-dependent and if it contributes to cell cycle progression.

Methods. Cells were synchronized in different phases of the cell cycle using □-factor peptide (G1 arrest), hydroxyurea (S arrest) and nocodazole (G2/M arrest). Cell cycle arrest or progression upon □-factor release were confirmed by analyzing DNA content by flow cytometry. The Atp2p levels were analyzed by western blot. The mitochondrial respiration was evaluated throughout cell cycle by measuring the oxygen consumption in a Clark electrode.

Results. We found Atp2p levels vary in accordance with cell cycle phases, with an increase at G1 and at G2/M. The increase in Atp2 protein levels at G2/M correlates with an increase in mitochondrial respiration, both reduced using an Atp2-T124A/T317A phosphoresistant mutant, indicating Atp2 phosphorylation plays a role in mitochondrial function at G2/M.

Since the two Atp2 phophosites lie within consensus motifs typically recognized by APC/C, an ubiquitin ligase with a role in cell cycle progression, we investigated if the phosphorylation increases Atp2p levels by preventing APC/C recognition. However, when mutating the putative APC/C recognition motifs, Atp2p levels did not increase. In addition, overexpression of both APC/C regulators, Cdh1 or Cdc20, did not decrease Atp2p levels, suggesting Atp2 is not an APC/C substrate. In addition, Atp2p levels were also not stabilized in a proteasome-deficient strain, suggesting Atp2 phosphorylation may contribute to Atp2 import/stabilization at the mitochondria.

To investigate if Atp2 phosphorylation plays a role in cell cycle progression, we monitored cell cycle kinetics in synchronized cells and found cell cycle progresses similarly in the Atp2-T124A/T317A mutant until G2/M when the transition to G1 is delayed. This effect is also observed by monitoring optical density during cell cycle.

Conclusions. Our results showed that Atp2 phosphorylation is associated with cell cycle, leading to increased mitochondrial respiratory activity at G2/M, which contributes for the cell cycle progression at this phase. These data suggest that Atp2 phosphorylation contributes to meet the energetic demands of cell cycle progression.

References.

[1] Pereira C et al. (2018) Biochim Biophys Acta Bioenerg 1859, 591601

This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., under the project UIDB/04293/2020, and through PhD grants attributed to ACL (SFRH/BD/135921/2018) and TSM (SFRH/BD/136996/2018) and FCT investigator grant attributed to CP (IF/00889/2015).

OC29 - Changes in salivary proteome in patients with dry mouth syndrome- the effect of pilocarpine therapy

Carreira. L[1, 4], Zamora. J[2], Nunes.J[2], Coromina. J[2], Cordeiro.C[3], Lamy, E[4]

[1]Biochemistry PhD student, Chemistry department, University of Évora, 7002-554 Portugal, [2] Dry mouth institute, Teknon Hospital, 08022 Spain; [3]FT-IRC and Structural Mass Spectrometry Laboratory, Faculty of Science, University of Lisbon, Campo Grande 1749-016, Lisbon, Portugal; [4]MED- Mediterranean Institute for Agriculture, Environment and Development; University of Évora, 7002-554 Portugal

Email address: d47128@alunos.uevora.pt

Background. Saliva is a biological fluid, mainly consisting of water, electrolytes, metabolites and proteins. Its function is coating and protecting surfaces of oral cavity, defending and lubricating them, facilitating bolus formation and swallowing. It is known that volume changes (e.g. hyposalivation) can cause dry mouth feeling, however, saliva composition changes cannot be excluded. Dry mouth syndrome, also called xerostomia, is a pathology characterized by a subjective dry mouth sensation. This syndrome can have variable origins, being the most common autoimmune diseases (e.g. Sjogrens' syndrome), poly-medicated patients, radiotherapy in the head and neck area or even idiopathic. Dry mouth causes oral discomfort, burning sensation, loss of taste, difficulties in chewing, among others. Existent treatments consist in strategies that improve symptoms, like topical medications, such as pilocarpine, the one focused in this study. Despite being known that pilocarpine induces increases in the flow of water and electrolytes to saliva, the aim of this pilot work was to access the effect of pilocarpine treatment in the salivary proteome of dry mouth syndrome patients.

To achieve this objective, protein quantification followed by electrophoretic separation was used. Saliva samples were collected to patients with Dry mouth syndrome attending the consultation of Instituto de la boca seca, Barcelona (N=7). Collections were made in two different days: 1) control situation, before treatment, when attending the first consult; 2) after a period of 3 to 9 months with pilocarpine treatment, through the administration of *Salagen* 5mg, three to five times a day, according to the severity of each patient symptoms. In all cases, saliva was collected by the passive droll method, to a tube maintained on ice and immediately frozen after collection, until laboratory analysis.

Methods. After assessing saliva protein concentration, through Bradford method, using bovine serum albumin as standard, saliva proteins were separated by SDS-PAGE, in 14% polyacrylamide mini-gels. Protein profiles of saliva obtained before and after pilocarpine treatment were compared.

Results. With this preliminary study we realized that the treatment with pilocarpine in continuous for a long period, result in changes in the protein profile. In fact, some of the protein bands presented differences in the expression levels, induced by pilocarpine treatment. These results will be presented and discussed in this presentation and suggest that, in opposition to some thoughts about pilocarpine as a drug inducing only changes in flow rate, this treatment also changes the protein composition of this fluid.

Conclusions. This potential association of the pathology with salivary proteome, reinforces the relevance continuing to explore saliva composition for a better understanding and treatment of this pathology.

OC30 - Exploration of the *Desulfuromonadales* "cytochromome". Purification of multiheme cytochromes involved in the extracellular respiration of *Desulfuromonas acetoxidans*

Ricardo M. Soares[1,2*], Catarina M. Paquete[1] and Ricardo O. Louro[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal, [2] Instituto Nacional de Investigação Agrária e Veterinária, Portugal

*Email address: ricardosoares@itqb.unl.pt

Background. The *Desulfuromonadales* order is composed by strict anaerobic bacteria, most with a great plasticity for respiration of soluble and insoluble electron acceptors. Apart from mesophiles and neutrophiles it also comprises thermophiles, halophiles and alkaliphiles. Some members of this order are also electroactive microorganisms (EM) that interact with electrical devices by transferring electrons to anodes when respiring anaerobically. Their power output and biofilm thickness in bioanodes are typically high when compared to other EM (e.g. *Shewanella* spp.). This has called the attention for their implementation in bioelectrochemical systems (BES), in particular for sustainable energy production, water desalination and bioremediation. Even though diversified bacteria compose the *Desulfuromonadales* order, only *Geobacter sulfurreducens* has been extensively studied and applied in this context. Since this bacterium was isolated from fresh water environments, power output in BES devices is greatly limited by the electrolyte (internal) resistance. For this matter, it is hypothesize that halophilic EM can enable an increase in conductivity in BES devices without affecting the physiology of the EM. Moreover, certain BES applications may imply the use of halophilic EM, e.g. water desalination. Here we aim to study the evolutionary patterns of the *Desulfuromonadales* with special emphasis on multiheme cytochromes *c* (MHC) and to unravel the extracellular electron transfer (EET) mechanisms of the marine bacterium *Desulfuromonas acetoxidans*.

Methods. To study the evolutionary patterns within the *Desulfuromonadales*, pan-genome analysis was performed by retrieving proteomes from NCBI and identifying *c*-type cytochromes with InterProScan. To test *D. acetoxidans* ability to grow in different conditions, growth curves were performed with different salt concentrations. EET under different salt concentrations was also assessed using methyl orange reduction colorimetric assays. MHC involved in EET were identified by homology with characterized MHC from EM. Their genes were amplified and cloned in appropriate expression vectors to be heterologously expressed in *Escherichia coli*. These proteins were purified by affinity chromatography and characterized biochemically.

Results. Pan-genome analysis revealed that *Geobacter* spp. contain the largest amount of coding sequences for MHC, while *Pelobacter* spp. the least. *Desulfuromonas* spp. genomes possess MHC coding sequences with the highest number of putative heme binding motifs per polypeptide. The best growth conditions of *D. acetoxidans* was achieved with 2% of NaCl, while the reduction of methyl orange was faster with 3% of NaCl. A putative EET pathway composed by MHC in *D. acetoxidans* was identified and purified. This proposed pathway is homologous to *G. sulfurreducens* EET pathway, being composed by homologues of CbcL, (inner membrane), cytochrome c_7 (periplasmic) and OmbacB (outer membrane cytochrome-porin complex).

Conclusions. Altogether, this work paves the way to study and apply new alternative EM that are able to power BES devices with high ionic strength conditions.

SYMPOSIUM 9 - SEBBM - CHEMICAL BIOLOGY, DRUG DISCOVERY AND DEVELOPMENT

Medicinal chemistry is a highly multidisciplinary and rapidly developing area that deals with the research on biologically active compounds and their interactions with biological targets to understand their mode of action at the molecular level, and how they can be included in clinically-relevant formulations. This session will provide insights into recent advances on different techniques for design and preparation of new drug leads in antiviral, antimicrobial and anticancer chemotherapy, as well as new drug candidates for contraception, neurodegenerative and inflammatory diseases. Emphasis will be focused on identification of multitarget-directed small molecules as innovative therapeutic tools, and best administration routes. Mechanism of action and structure–activity relationship studies, including elucidation of target enzyme/protein-drug interaction and metabolic pathways will be discussed.

IL16
Ana Mata Duran (UEx, Spain)

IL17
João Ramalho Santos (CNC-UC)

INVITED LECTURES S9

(IL16-IL17)

Ana Mata Duran
Department of Biochemistry and Molecular Biology, Faculty of Sciences and Institute of Molecular Pathology Biomarkers, University of Extremadura, Badajoz, Spain.

The emerging role of methylene blue in the interplay between PMCA and biomarkers of Alzheimer's disease

João Ramalho-Santos
Department of Life Sciences, University of Coimbra, Portugal & Center for Neuroscience and Cell Biology, University of Coimbra

I want a new drug: Searching for novel modulators of sperm function

Chairs: Carlos Gutierrez-Merino (UEx, Spain); Manuel Aureliano (UAlg)

IL16 – The emerging role of methylene blue in the interplay between PMCA and biomarkers of Alzheimer's disease

Ana M. Mata

Department of Biochemistry and Molecular Biology, Faculty of Sciences and Institute of Molecular Pathology Biomarkers, University of Extremadura, Badajoz, Spain.

Email address: anam@unex.es

Calcium (Ca^{2+}) is a major signalling ion which regulates many cellular functions. Therefore, the low resting intracellular Ca^{2+} concentration and the Ca^{2+} signal have to be strictly regulated. Among all systems involved, the plasma membrane Ca^{2+}-ATPase (PMCA) is a high-affinity active Ca^{2+} transporter which extrudes the excess of intracellular Ca^{2+} out of the cell. Dysregulation of intracellular Ca^{2+} homeostasis is a key factor in brain aging and aging-associated neurodegenerative diseases, such as Alzheimer´s disease (AD). This disease is characterized by the presence of amyloid plaques composed of amyloid β-peptide (Aβ) and neurofibrillary tangles of abnormal tau protein. We have shown that PMCA activity is inhibited by Aβ and tau, and we have characterized in detail the inhibitory mechanisms. In order to look for compounds that could block those inhibitions we have analyzed the effects of methylene blue, a phenothiazine which have been reported to ameliorate mild cognitive impairment and mild AD. Our results indicate that methylene blue prevents and even blocks the inhibition of PMCA activity by Aβ and tau, through interactions with the pump and also with the inhibitors. Based on these results we suggest that one of the molecular mechanisms underlying a potential therapeutic effect of methylene blue in AD may involve the regulation of intracellular Ca^{2+} levels by its interaction with PMCA, Aβ and tau and with the complex PMCA-Aβ and PMCA-tau.
Funding: Supported by Spanish grants from Ministerio de Economía y Competitividad (MINECO, BFU201785723P) and Junta de Extremadura (GR18118), cofinanced by FEDER funds.

IL17 – I want a new drug: Searching for novel modulators of sperm function

João Ramalho-Santos[1,2]

[1]Department of Life Sciences, University of Coimbra, Portugal [2]CNC_ Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Email address: jramalho@uc.pt

Mammalian sperm function is a complex process that involves many different pathways and aspects, not only related to the obvious readout related to motility, but also in terms of other processes needed for the sperm to correctly fertilize an oocyte, and that go well beyond merely reaching the female gamete. Systematically studying these events can ultimately provide clues to manipulate sperm production and functionality, although one must consider that a mature sperm cell is devoid of many of the main features relevant for somatic cell function, and its manipulation using strategies normally applied to other cells may be therefore prove technically demanding. Besides invasive strategies related to modulating sperm production in the testis, this leaves biochemical modulation of the male gamete itself.

Manipulating human sperm function may serve two distinct purposes. On one hand, potentiating sperm activity in some cases of male subfertility/subfertility may render cells more amenable to natural conception or for certain Assistance Reproduction techniques. On the other hand, impairing sperm function may be relevant in developing novel male-tailored contraception strategies. In the latter case the specificity of whatever compounds proposed to be employed solely towards sperm, as well as lack of unspecific toxicity of these same compounds to the female reproductive tract are the main concerns and need to be carefully controlled. The talk will review several strategies related to the biochemical manipulation of human sperm with relevance towards contraception and Assisted Reproduction.

ORAL COMMUNICATIONS S9

OC31	João Nunes	Biocompatibility and biosafety analysis of chitosan hydrogels using organotypic epidermal models
OC32	Ana Salomé Veiga	Anti-HIV-1 activity and mode of action of pepRF1, a viral-derived CXCR4 antagonist
OC33	Vera Neves	Development of a Blood Brain Barrier Peptide Shuttle and its conjugation to an Fc domain for brain delivery of therapeutic biomolecules
OC34	Andreia Veloso	Development of a highly hydrophilic carbon SOD-nanozyme

Chairs: Carlos Gutierrez-Merino (UEx, Spain); Manuel Aureliano (UAlg)

OC31 - Biocompatibility and biosafety analysis of chitosan hydrogels using organotypic epidermal models

Nunes J.[1], Marques M.[2], Sterghite D[2]., Branco S.[3,4], Martins L.[3,4], Silva M.[5], Campos-Gonçalves I.[5] Souza E.F[5], Filho C.M.C.[5], Craveiro A.C.[5], Alpizar-Jara R.[6,7], Burke A.J.[1,8], Costa A.R.[2,9], Antunes C.M.[2,9]

[1]Department of Chemistry, School of Sciences and Technology, University of Évora, Portugal; [2]Institute of Earth Sciences – ICT; [3]Mediterranean Institute for Agriculture, Environment and Development – MED; [4]Department of Veterinary Medicine, School of Sciences and Technology, University of Évora, Portugal; [5]BRinova Biochemistry Lda., Évora, Portugal; [6]Research Center in Mathematics and Applications – CIMA; [7]Department of Mathematics, School of Sciences and Technology, University of Évora, Portugal; [8]Institute for Research and Advanced Learning – LAQV; [9]Department of Health and Medical Sciences, School of Health and Human Development, University of Évora, Portugal.

Email address: jpedropnunes@gmail.com

Background: Frequent dog consultations are due to skin wounds (Holland, 2019), with different trauma causes, like bites, burns and others. The majority of these wounds are not fatal, but cause long-lasting discomfort, demanding frequent medical care which may affect owners financially (Fahie & Shettko, 2007). Despite several pharmaceuticals available for dog wound treatment, new antibiotic-free efficient options, also more ecological are needed. Chitosan hydrogels offer a promising solution. Besides, being very ecological and biodegradable, chitosan hydrogels allow the maintenance of a moist environment that assists the exchange of fluids, essential for wound healing, and can also incorporate agents to avoid the development of infectious agents (Stashak, Farstvedt, & Othic, 2004). The aim of this study was to screen chitosan-based hydrogels for veterinary applications, supplied by the company BrInova Biochemistry as part of a collaborative project (NAQUIBIO DPSA). Screening was based on toxicity, biosafety, and efficacy "in vitro" tests, using organotypic epidermal models.

Methods: Epidermal canine keratinocytes and human fibroblasts primary cells (supplied by CELLnTEC) were seeded in flasks and incubated to reach a desired level of growth, as indicated by the supplier. For toxicity evaluation, both cell lines were used. Viability tests were performed in 96 wells cell-culture plaques. Culture media and Triton X-100 was used as negative and positive controls, respectively. Firstly, hydrogels and potentially useful additives were tested individually, in several concentrations. Nontoxic components and doses were used in the production of several hydrogel-based composites, subsequentially tested. Viability was accessed by dehydrogenase activity (CCK-8, Sigma-Aldrich). For the efficacy of the hydrogel-based composites a wound scratch (WS) cellular assay was used, applied to both cell lines. For biosafety tests, a 3D keratinocyte culture was prepared, and irritation, corrosion, and sensitization protocols (adapted of EPISKIN™) were used.

Results: Toxicity testes allowed the selection of 4 hydrogels, 2 nanoparticles and 2 plant essential oils to use in hydrogel-based composites, as well as the suitable concentrations range for each. Based on that information, 16 composites were prepared by other members of the NAQUIBIO team, and their toxicity was also evaluated, allowing the selection of 6 hydrogel-based composites. Those composites were evaluated for efficacy by a WS assay and the best compounds were those that could induce a fast closure of the scratch gap. The intersection of the toxicity and efficacy results allowed the selection of one hydrogel-based composite as the most promising one, whose biosafety was later evaluated. This proved to be non-corrosive, non-toxic and non-sensitizing. This compound was selected to proceed to the animal testing phase, in accordance with the goals of the NAQUIBIO project.

Conclusions: As initially planned, we were able to select a mixture of hydrogels with essential oils and nanoparticles, to be further *in vivo* evaluated in dogs, or more specifically we have in advanced development a new wound dressing for veterinary use, capable of accelerating and creating better conditions for wound healing.

References.

Fahie, M. A., & Shettko, D. (2007). Evidence-Based Wound Management: A Systematic Review of Therapeutic Agents to Enhance Granulation and Epithelialization. *Veterinary Clinics of North America - Small Animal Practice*, *37*(3), 559–577. https://doi.org/10.1016/j.cvsm.2007.02.001;

Holland, K. E. (2019). Acquiring a pet dog: A review of factors affecting the decision-making of prospective dog owners. *Animals*, *9*(4), 1–18. https://doi.org/10.3390/ani9040124;

Stashak, T. S., Farstvedt, E., & Othic, A. (2004). Update on wound dressings: Indications and best use. *Clinical Techniques in Equine Practice*, *3*(2 SPEC. ISS.), 148–163. https://doi.org/10.1053/j.ctep.2004.08.006

Acknowledgments.. This work was co-funded by the Project NAQUIBIO DPSA - Obtenção de Medicamentos Veterinários a partir de Nanopartículas Prata fixadas em Bases Poliméricas de Hidrogéis de Quitosana, Alentejo 2020, Co-Promotion, Ref. 03/SI/2017 (ALT20-03-247-FEDER-033578) Co-Financed by FEDER - COMPETE 2020. FEDER Funds through the Operational Programme for Competitiveness Factors – COMPETE and National Funds through FCT – Foundation for Science and Technology under the Project UIDB/50006/2020. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020

OC32 - Anti-HIV1 activity and mode of action of pepRF1, a viral-derived CXCR4 antagonist

Cadima-Couto I.[1], Tauzin A.[2], Freire J.M.[1], Figueira T.N.[1], Silva R.D.M.[3], Pérez-Peinado C.[4], Cunha-Santos C.[5], Bártolo I.[5], Taveira N.[5,6], Gano L.[3], Correia J.D.G.[3], Goncalves J.[5], Mammano F.[2], Andreu D.[4], Castanho M.A.R.B.[1], Veiga A.S.[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;; [2]INSERM UMR 1124, Université de Paris, Paris, France; [3]Centro de Ciências e Tecnologias Nucleares and Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal; [4]Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain; [5]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [6]Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Universitário Egas Moniz, Monte de Caparica, Portugal

Email address: aveiga@medicina.ulisboa.pt

Background. There is an urgent need for the development of new anti-HIV drugs that can complement existing medicines to be used against resistant strains. Here, we report the anti-HIV-1 peptide pepRF1, a human serum-resistant peptide derived from the Dengue virus capsid protein.

Methods. Infectivity and virus-cell fusion assays using lab-adapted HIV-1 strains, patient-derived viruses, and a T20-resistant virus, were used to study the anti-HIV activity of the pepRF1. Flow cytometry- and fluorescence-based assays were used to investigate the mechanism of action of the peptide.

Results. *In vitro*, pepRF1 shows a 50% inhibitory concentration (IC_{50}) of 1.5 nM with a potential therapeutic window higher than 53,000. This peptide is specific for CXCR4-tropic HIV-1 strains, preventing viral entry into target cells by binding to the viral co-receptor CXCR4. Upon binding, neither internalization nor intracellular Ca^{2+} influx are triggered, showing that pepRF1 is an antagonist of this chemokine receptor. pepRF1 is more effective than T20, the only peptide-based HIV-1 entry inhibitor approved by FDA for clinical use, and excels in inhibiting an HIV-1 strain resistant to T20 (HIV-1NL4.3 DIM) with an IC_{50} of 2.8 nM.

Conclusions. Overall, our study led to the discovery of a peptide highly active against HIV-1 that acts as a CXCR4 antagonist. Potentially, pepRF1 can be used alone or in combination with other anti-HIV drugs to fight AIDS. Furthermore, one can also envisage its use as a novel therapeutic strategy for other CXCR4-related diseases.

OC33 - Development of a Blood Brain Barrier Peptide Shuttle and its conjugation to an Fc domain for brain delivery of therapeutic biomolecules

Cavaco, M.[1,2], Frutos, S.[3], Oliete, P.3, Valle, J.[2], Andreu, D.[2], Castanho, M. A. R. B. [1], Vila-Perelló, M.[3], and Neves, V.[1].

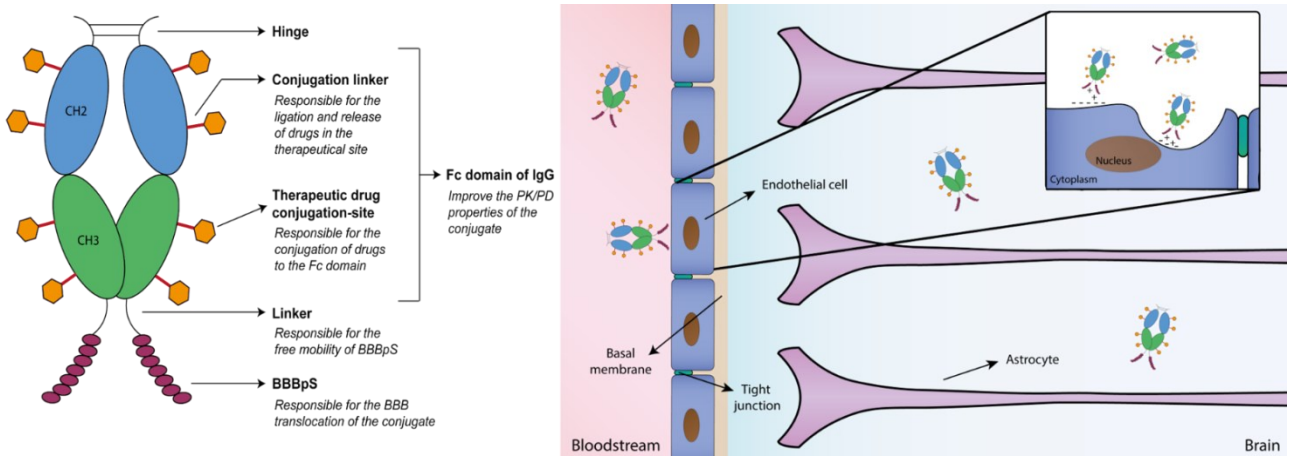
[1]Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Av. Prof Egas Moniz, 1649 – 028 Lisboa, Portugal; [2]Proteomics and Protein Chemistry Unit, Department of Experimental and Health Sciences, Pompeu Fabra University, Dr. Aiguader 88, Barcelona Biomedical Research Park, 08003 Barcelona, Spain; [3]SpliceBio S.L., Baldiri Reixac 10 – 12, 08028 Barcelona, Spain;

Email address: veraneves@medicina.ulisboa.pt

The frequency of brain diseases has increased significantly in the past years. After diagnosis, therapeutic options are usually limited, which demands the development of innovative therapeutic strategies. The use of antibody-drug conjugates (ADCs) is promising but highly limited by the existence of the blood-brain barrier (BBB) [1]. To overcome the impermeability of this barrier, antibody fragments can be engineered to contain a BBB peptide shuttle (BBBpS), capable of brain penetration. PepH3, a seven-residue sequence derived from the α-helical domain of the dengue virus type-2 capsid protein, and its enantiomer D-PepH3, efficiently translocate across the BBB using a receptor-free mechanism [2]. In addition, these BBBpS presents very low toxicity and stability (D-PepH3). Here, we linked PepH3, to the IgG fragment crystallizable (Fc) domain using streamlined expressed protein ligation (SEPL) method. With this strategy, we obtained an Fc-PepH3 scaffold that can carry different payloads. Fc-PepH3 was shown to be non-toxic to brain endothelial cells and red blood cells (RBCs). Fc-PepH3 is capable of crossing an in vitro cellular BBB model, with a percentage of translocation 2.4-fold higher than unconjugated Fc. In addition, Fc-PepH3 binds to neonatal Fc receptor (FcRn), which is responsible for antibodies long half-life (t1/2) at pH 6.0. The determined binding affinity was 95.4±9.21 nM for Fc-PepH3, which is comparable to unconjutated Fc (89.6±8.78 nM). Overall, we demonstrated the potential of Fc-PepH3 as a versatile platform readily adaptable to diverse drugs to treat different brain conditions.

References

1. Cavaco, M., et al. Peptibodies: An elegant solution for a long-standing problem. Peptide Science 2018, 110 (1), e23095.
2. Cavaco, M., et al. DPepH3, an Improved Peptide Shuttle for Receptor-independent Transport Across the Blood-Brain Barrier. Current Pharmaceutical Design 2020, 26, 1-12.



OC34 - Development of a highly hydrophilic carbon SOD-nanozyme

Veloso A. D.[1], Oliveira, M. C.[1], Videira R. A[2]

[1] CQ-VR e Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal;
[2]REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

Email address: andreiaveloso8@hotmail.com

Superoxide dismutases (SODs), a family of metalloenzymes that catalyze the dismutation of superoxide anion radical into molecular oxygen and hydrogen peroxide, are key players in the regulation of the cellular redox balance and cell signaling cascades. SOD deficiencies/anomalies have been associated with disparate human diseases, which exhibit oxidative damage and chronic inflammation as common pathological hallmarks. Thus, the development of nanomaterials with catalytic activity similar to native enzymes to overcame SOD deficiencies became a great challenge of nanomedicine. To address this challenge, we propose a water-soluble carbon-based nanomaterial produced galvanostatically from graphite in a phosphate buffer (EHC@phosphate). EHC@phosphate exhibits a structure dominated by sp^2 carbons in a non-order carbon network formed by small clusters (< 2 nm) of carbonaceous material with many oxygen-based functional groups, including quinone, epoxide, and hydroxyl groups. Using cyclic voltammetry, it was shown that EHC@phosphate displays a reversible redox pair with a formal potential at -0.05 V (vs Ag/AgCl) and an irreversible reduction peak at -0.550 V (vs Ag/AgCl). The EHC@phosphate reversible redox pair is close to the reduction potentials exhibited by the native catalytic centers of the different isozyme forms of SOD, suggesting the potential to promote reversibly both half-reactions of the superoxide anion radical dismutation. The SOD-like activity was evaluated using the hypoxanthine-xanthine oxidase system as the generator of superoxide anion radical and the reduction of nitro blue tetrazolium chloride (NBT) as detector system. The kinetics of the reduction of the superoxide anion radical detection was characterized as a function of the EHC@phosphate nanomaterial concentration, and results compared with commercially available graphene quantum dots (GQDs) and graphene oxide (GO) as well as with a native SOD enzyme. In opposition to GQDs and GO that do not exhibit SOD-like activity under physiological-like conditions, the EHC@phosphate nanomaterial (0-15 ug/mL) exhibits a remarkable SOD-like activity. A mechanism for the dismutation of superoxide anion radical by the EHC@phosphate nanomaterial is proposed considering cyclic redox changes compatible with nanomaterial functional groups and structural features. Preliminary cell viability studies showed that EHC@phosphate nanomaterial displays no toxicity for human neuronal SH-SY5Y cells. Thus, EHC@phosphate emerges as a new SOD-mimic nanomaterial with the potential to be considered for therapeutic applications development.

Acknowledgments. The authors thank FCT (Portugal's Foundation for Science and Technology) for financial support through the CQ-VR (UIDB/00616/2020, UIDP/00616/2020) and REQUIMTE/LAQV (UIDB/50006/2020) Research Units projects. Andreia D. Veloso thanks FCT, MCTES, ESF, and EU for the individual research Ph.D. scholarship (SFRH/BD/138425/2018).

FLASH COMMUNICATIONS/POSTER PRESENTATIONS

S1 – MOLECULAR MECHANISMS OF DISEASE

FC1	André Seixas	The oxidative stress response of Listeria monocytogenes is mediated by the RNA chaperone Hfq
FC2	Carolina Matos	Development of antibodies targeting the Notch ligand JAG1 with potential efficacy against aggressive cancers
FC3	Maria Firmino	Unraveling genetic and pathogenic characteristics of infectious Shewanella
FC4	Bárbara Matos	Chronic exercise training attenuates prostate cancer-induced molecular remodelling in the testis
FC5	Tatiana Varela	Characterization of zebrafish cdkl5 knockdown using a morpholino approach
FC6	Barbara Rocha	Inorganic nitrate prevents the loss of intestinal claudin-5 induced by broad-spectrum antibiotics but has a mild impact on gut microbiome diversity: may nitrate be fueling bacterial metabolism?
FC7	Catarina Sousa Lopes	Changes on fibrinogen-erythrocyte binding in carotid artery disease
FC8	Telma Martins	Vacuolar membrane proteome and role of amino acids in the lifespan of a Niemann-Pick type C1 yeast model

Chairs: Manuel Santos (UAv); Álvaro Tavares (UAlg)

FC1 - The oxidative stress response of *Listeria monocytogenes* is mediated by the RNA chaperone Hfq

Seixas, A., Silva, A., Arraiano, C.M. and Andrade, J.M.

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Avenida da República 2780-901, Oeiras, Portugal.

Presenting author: andre.seixas@itqb.unl.pt

Background. The RNA chaperone Hfq is an important bacterial post-transcriptional regulator. In Gram-negative bacteria like *E. coli* and *Salmonella*, it is widely known to promote the base-pairing between non-coding small RNA and mRNA. However, in Gram-positive bacteria like *Listeria monocytogenes*, Hfq main role remains elusive as this protein seems to be expendable for this interaction.

Methods. To investigate the role of Hfq in the human pathogen *Listeria monocytogenes*, we used a combined approach using microbiology and biochemistry tools. We tested the requirement of Hfq facing an array of stressors in rich culture medium. Through an enzymatic activity assay, we prove that Hfq affects catalase activity in crude extracts. RNA analysis techniques (qPCR and Northern blot) confirmed that Hfq is a new regulator of catalase expression.

Results. We found that Hfq is essential for the oxidative stress response of *Listeria*, with disruption of the *hfq* gene resulting in a hypersensitive phenotype to hydrogen peroxide (H_2O_2).

1. Using a sub-inhibitory concentration of H_2O_2, the growth of the wild-type strain was barely affected and recovered immediately upon addition of the oxidative stressor. However, the *hfq* null-mutant culture could not resume growth. This growth inhibition after stress was further confirmed in solid medium, with the *hfq* mutant showing reduced viability upon exposure to H_2O_2 when compared to the wild-type strain.

2. H_2O_2 is one of the several reactive oxygen species (ROS) found inside cells, and H_2O_2 decomposition is mainly mediated by catalase. An enzymatic activity assay demonstrated that catalase is significantly less active in the absence of Hfq when compared to the wild type.

3. We further demonstrate that Hfq is a novel regulator of catalase expression, by regulating catalase mRNA levels, specifically under oxidative stress conditions.

4. In addition, an infection biology assay revealed that the *hfq* mutant is more susceptible to the action of macrophages, whose primary source of defence is the production of ROS species.

Conclusions. The results obtained here associates, for the first time, Hfq and the way *Listeria* copes with oxidative stress. This may contribute to understand novel regulatory pathways controlling the oxidative stress response of bacterial pathogens and uncover novel strategies to fight ROS species in the infection process of intracellular bacteria.

FC2-Development of antibodies targeting the Notch ligand JAG1 with potential efficacy against aggressive cancers

Carolina Matos[1], Gonçalo Marques[1], Rute Eleutério[1], Khrystyna Kucheryava[1], Ana R. Lemos[1,2], Ana Barbas[1,3], Pedro M. F. Sousa[1,2], Rute Castro[1], Gabriela Silva[1]

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Portugal. [2]Instituto de Tecnologia Química e Biológica António Xavier, Portugal.3 Bayer Portugal, Portugal.

E-mail address: carolina.matos@ibet.pt

Background. The Notch signaling ligand JAG1 is a key oncogene in subsets of aggressive breast, ovarian, prostate, pancreatic, and stomach cancers and correlates with poor clinical prognosis. JAG1 overexpression in these tumors contributes to tumor growth, metastasis, recurrence and drug resistance by promoting cancer cell survival, proliferation, migration, invasion, cancer stem cell function, tumor angiogenesis and anti-tumor immunity (*1, 2*). These multiple roles of JAG1 in tumor biology support the development of specific anti-JAG1 therapies for the treatment of aggressive tumors, which constitute a major health concern. JAG1-targeting therapeutics are expected to provide clinical benefit as monotherapy and in combination with chemotherapy by impairing therapy-enriched cancer stem cells, reducing metastasis and relapse. Antibodies (Abs) are an important component in the arsenal of cancer therapeutics (*3*). In this study we aim to develop an anti-JAG1 Ab with anti-tumor efficacy.

Methods. Antibody fragments against human JAG1 were selected by phage display technology using recombinant human JAG1 protein (rhJAG1) as antigen. Unique fragments specifically binding to rhJAG1 were converted to complete Ab molecules by cloning their variable heavy and light regions in two distinct mammalian expression vectors encoding the human IgG1 constant regions for light chain (LC) and heavy chain (HC). To produce anti-JAG1 Abs, HEK293E6 suspension cells were transfected with the respective LC and HC plasmids. Secreted Abs were purified from culture media in endotoxin-free conditions using protein-A affinity and size exclusion chromatography. Binding ability and specificity of anti-JAG1 Abs were tested by ELISA, surface plasmon resonance, and flow cytometry.

Results.1- Seven unique JAG1 Ab fragments were selected and converted into 7 anti-JAG1 Abs. 2- Purified Abs have the expected size, purity >95% and low endotoxin content. 3- Two Abs bind rhJAG1 with low nanomolar affinities while 3 Abs showed moderate binding and 2 Abs low binding. 4- All Abs are specific for JAG1 since they do not bind to the other human Notch ligands. 5- JAG1 Abs bind JAG1-overexpressing cells and different cancer cells endogenously expressing JAG1 but not to cells without JAG1 surface expression. 6- Specific binding of anti-JAG1 Abs to cell surface JAG1 was confirmed using a JAG1 knockout cell line we generated using the CRISPR/CAS9 system.

Conclusions. We were able to select specific anti-JAG1 Abs, 2 of them with good kinetic affinities that specifically bind to cellular JAG1. Cellular assays are warranted to explore their anti-tumor efficacy.

References.

1. D. Li, M. Masiero, A. H. Banham, A. L. Harris. *Frontiers in Oncology* **4**, 254 (2014).
2. M. X. Xiu, Y. M. Liu, B. H. Kuang. *Biomed Pharmacother* **129**, 110416 (2020).
3. R. M. Lu *et al.*, *Journal of Biomedical Science* **27**, 1 (2020).

FC3-Unraveling genetic and pathogenic characteristics of infectious *Shewanella*

Firmino, M. O.[1,2], Silva, A.V.[1], Louro, R.O.[1], Ramirez, M.[2], Paquete, C. M.[1]

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. Da República – EAN, 2780 – 157 Oeiras, Portugal 1, [2]Instituto de Microbiologia da Faculdade de Medicina da Universidade de Lisboa, Avenida Prof. Egas Moniz, P-1649-028 Lisboa.

Email address: mfirmino@itqb.unl.pt

Background *Shewanella* spp. are ubiquitous bacteria, commonly used in biotechnology[1]. Although this genus is usually isolated from non-human sources, it has recently been identified as an emerging opportunistic pathogen[1,2]. Reported cases of *Shewanella putrefaciens* infections are increasing, mostly skin and soft tissue infections, but also meningitis, otitis media and bacteraemia[3]. The mis-identification of *Shewanella* species is common, when using the conventional identification systems available in routine clinical microbiology laboratories[4]. By genomic analysis, this work aims to confirm the identification of *S. putrefaciens* of isolates responsible for infection, genes potentially encoding virulence factors and to evaluate their role in pathogenesis in an animal model.

Methods To achieve this, the first step was the sequencing of *S. putrefaciens* DSM 9451, isolated from a human infection, using high throughput sequencing (Illumina NextSeq). The genome was *de novo* assembled and annotated using the INNUCA pipeline and genes of interest were identified using relevant databases, such as VFBD and Resfinder.

Results The analysis of the genome strongly suggests a misclassification of this isolate and the existence of a novel species. Other strains isolated from infection and for which HTS data is available also seem to belong to this novel species. Moreover, genome analysis showed the presence of genes considered to be involved in virulence, such as *flip* and *luxS*, as well as genes that are responsible for antibiotic resistance, such as blaOXA-436 and *tet*(G). Other genes, such as *bolA*, responsible for biofilm formation[5], and *sip* and *fsr*, important for iron scavenging[6], are also considered to encode virulence factors[5,6], and present in the genome of DSM 9451. To test the importance of these genes in the pathogenic process, phenotypic and infections studies in an animal model using knock-out strains[7] are being conducted.

Conclusions *S. putrefaciens* causing human infections seem to represent a novel genomovar which can potentially be considered a novel species. These isolates carry several potential virulence genes being present in these isolates.

References

1. Janda, J. M. & Abbott, S. L. The genus Shewanella: From the briny depths below to human pathogen. *Crit. Rev. Microbiol.* **40**, 293–312 (2014).
2. Janda, J. M. Shewanella: A Marine Pathogen as an Emerging Cause of Human Disease. *Clin. Microbiol. Newsl.* **36**, 25–29 (2014).
3. Tena, D., Losa, C., Carrasco, G. & Sáez-Nieto, J. A. Surgical Site Infection Caused by Shewanella putrefaciens. *Infect. Dis. Clin. Pract.* **24**, 18–23 (2016).
4. Hochedez, P. *et al.* Human Infection with Shewanella putrefaciens and S . algae : Report of 16 Cases in Martinique and Review of the Literature. **89**, 151–156 (2013).
5. Mil-homens, D. *et al.* Stress Response Protein BolA Influences Fitness and Promotes Salmonella enterica Serovar Typhimurium Virulence. *Genet. Mol. Biol.* **84**, 1–13 (2018).
6. Tu, J. *et al.* The siderophore-interacting protein is involved in iron acquisition and virulence of Riemerella anatipestifer strain CH3. *Vet. Microbiol.* **168**, 395–402 (2014).
7. Silva, A. V, Edel, M., Gescher, J. & Paquete, C. M. Exploring the Effects of bolA in Biofilm Formation and Current Generation by Shewanella oneidensis MR-1. *Front. Microbiol.* **11**, 1–7 (2020).

FC4-Chronic exercise training attenuates prostate cancer-induced molecular remodelling in the testis

Matos, B.[1], Patrício, D.[1] , Henriques, M.C.[1] , Freitas, M.J.[1] , Vitorino, R.[1] , Duarte, I.F.[2] , Howl, J.[3] , Oliveira, P.[4] , Seixas, F.[5] , Duarte, J.A.[6] , Ferreira, R.[7] , Fardilha, M.[1]

[1]Signal Transduction Laboratory, Institute of Biomedicine - iBiMED, Department of Medical Sciences, University of Aveiro, Portugal; [2]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal; [3]Molecular Pharmacology Group, Research Institute in Healthcare Science, University of Wolverhampton, UK, [4]CITAB, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [5]CECAV, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal, [6]CIAFEL, Faculty of Sport, University of Porto, Porto, Portugal; [7]QOPNA & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Portugal

Email address: barbaracostamatos@ua.p

Background. Prostate cancer (PCa) is a disquieting cause of cancer death worldwide and, in addition to impairing prostate function, also causes testis adaptations. Exercise training (EX) has been associated with a beneficial effect in both prevention and outcomes of PCa. Regarding the effect of EX in testis function there is no consensus in the literature, but a positive effect has been suggested in preventing or counteracting the impairment of testis function caused by several conditions. In this study, we investigated the preventive effect of EX in PCa-induced testicular dysfunction.

Methods. As a model, we used fifty Wistar Unilever male rats, randomly divided in four experimental groups (CONT+SED, PCa+SED, CONT+EX, PCa+EX). Prostate cancer was chemically and hormonally induced in two groups of animals (PCa groups). One control group (CONT) and one PCa group were submitted to moderate intensity treadmill exercise training. Fifty weeks after the start of the training the animals were sacrificed and sperm, prostate, testes and serum were collected and analysed. Sperm concentration and morphology, and testosterone serum levels were determined. In addition, histological analysis of the testes was performed, and testis proteomes and metabolomes were characterized.

Results. We found that prostate cancer negatively affected testicular function, manifested as an arrest of spermatogenesis. Oxidative stress-induced DNA damage, arising from reduced testis blood flow, may also contribute to apoptosis of germ cells and consequential spermatogenic impairment. An altered metabolism characterized by glycolysis downregulation, increased ketone bodies and glycogen metabolization, together with accumulation of branched chain amino acids, seems to energetically support testis function in PCa animals. Fifty weeks of moderate intensity treadmill EX prevented PCa-induced testis remodeling to some extent, mainly through the activation of DNA repair mechanisms, along with a recovery of the glycolytic pathway and the attenuation of PCa-induced increases in ketone bodies metabolization, despite no impact on oxidative stress. **Conclusions.** These findings confirm a negative impact of prostate cancer on testis function and suggest a beneficial role for exercise training in the prevention of prostate cancer-induced testis dysfunction

FC5-Characterization of zebrafish *cdkl5* knockdown using a morpholino approach

Varela T[1,2], Varela D[1,2], Martins G[1,2], Conceição N[1,3,4], Cancela ML[1,3,4]

[1]Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal; [2]PhD in Biomedical Sciences, Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal; [3]Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal; 4Algarve Biomedical Centre, University of Algarve, Faro, Portugal

Email address: a44770@ualg.pt

Background. Cyclin-dependent kinase-like 5 (*CDKL5*) gene encodes a serine/threonine kinase essential for normal brain development and function, being involved in important neuronal processes such as cell signaling and neuron morphogenesis. It is responsible for its autophosphorylation as well as the phosphorylation of its substrates including MECP2, DNMT1 and AMPH. Mutations in *CDKL5* are responsible for the CDKL5 deficiency disorder (CDD), a rare X-linked neurodevelopmental condition characterized primarily by seizures, which generally begin in the first months of life, and impairment of cognitive and motor skills. The association between the type/location of mutations and patient's phenotype, as well as the mechanisms responsible for its onset remain poorly understood. Zebrafish is an accepted biomedical model to study many genetic diseases including neurologic disorders and has many advantages over other models, thus being chosen to further study CDD. The aim of this work was to investigate the phenotypic and molecular consequences of a morpholino (Mo)-mediated *cdkl5* knockdown in zebrafish, to contribute to unveil mechanism of action and targets of Cdkl5.

Methods. To accomplish our objective, 1-cell stage zebrafish embryos were injected either with translation blocking morpholino (AUG Mo) to transiently suppress Cdkl5 expression or with control standard morpholino (Ctrl Std Mo). Total RNA of 3 dpf injected larvae was extracted and qPCR was performed to investigate expression levels of neuronal marker genes. The motor behavior was analyzed by the swimming activity of 5dpf larvae treated or not with PTZ, a seizure-inducing drug·

Results: Knockdown of *cdkl5* using AUG Mo resulted in zebrafish larvae with altered morphology in a dose dependent manner. At 3dpf, neuronal marker genes of larvae injected with 15 ng of AUG Mo were dysregulated in comparison with larvae injected with Ctrl Std Mo. Levels of *npy* were upregulated while levels of *mecp2*, *mef2ca*, *mef2cb* and *drd2c* were all downregulated. Locomotor activity of larvae injected with 15 ng of AUG Mo showed a significant decrease in the distance travelled comparing to the larvae injected with Ctrl Std Mo. Upon treatment with PTZ, a seizure behavior and an increase in the distance travelled was observed for both groups of larvae.

Conclusions. In conclusion, Cdkl5 ablation affects the expression of genes involved in neuronal processes and leads to the impairment of the motor activity of zebrafish larvae, a phenotype analogous to what is observed in CDKL5 deficiency disorder.

References

Fehr et al. (2013) The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. Eur J Hum Genet 21:266-273; Russo et al. (2009) Novel mutations in the CDKL5 gene, predicted effects and associated phenotypes. Neurogenetics 10:241-250

Funding. Million Dollar Bike Ride Grant program from the Orphan Disease Center, University of Pennsylvania, USA. And Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020. Varela T, Varela D and Martins G are recipient of PhD fellowship from FCT.

FC6-Inorganic nitrate prevents the loss of intestinal claudin-5 induced by broad-spectrum antibiotics but has a mild impact on gut microbiome diversity: may nitrate be fueling bacterial metabolism?

Rocha, B.S., Barbosa, R.M. and Laranjinha, J.

Faculty of Pharmacy University of Coimbra and Center for Neuroscience and Cell Biology, Coimbra, Portugal

Email address: barbarasrocha@ff.uc.pt

Background. Dietary nitrate is a redox signaling molecule with critical physiological functions both in the gut and systemically. In the distal bowel, nitrate may interact with the local microbiota, modulating not only the structure and function of local bacterial communities but also the epithelial barrier function. Although some data has been emerging on the effect of nitrate on oral microbiota, its impact on intestinal bacteria remains elusive. This study investigates the impact of nitrate on intestinal microbiota and the expression of local tight junction proteins.

Methods. Rats were divided in 4 groups and exposed to the following regimens for 7 days: 1) antibiotics, 2) antibiotics+nitrate, 3) nitrate and 4) tap water. Occludin and claudin-5 were analyzed by immunoblotting in the colon. Nitrate and nitrite were measured in intestinal tissue by HPLC and fecal bacterial DNA was studied by DGGE before and after treatment.

Results. Nitrate increases claudin-5 expression in rats exposed to a therapeutic dose of broad spectrum antibiotics in comparison to animals exposed to antibiotics alone ($p=0.016$) but decreases the expression of occludin ($p=0.003$), suggesting that different proteins may be modified by different mechanisms by nitrate. As expected, dietary nitrate increases intestinal nitrate concentration ($p=0.038$). In the presence of antibiotics, dietary nitrate increases tissue nitrate concentration by c.a. sixfold in comparison to both controls and rats exposed to antibiotics without supplementation ($p<0.0001$). Antibiotics eradicated most of gut flora ($p=0.0016$), reducing microbiota richness by 56% while nitrate showed a tendency to attenuate such microbial loss (48%, $p=0.068$).

Conclusion. Although nitrate consumption may be recommended during antibiotherapy, functional studies are mandatory to ascertain the impact of this anion on intestinal barrier function and bacterial metabolic pathways, which may recycle this anion and likely trigger different redox signaling pathways along the gut.

FC7-Changes on fibrinogen-erythrocyte binding in carotid artery disease

Catarina S. Lopes[1], Ryan Gouveia e Melo[2], Sofia Nabais[1], Teresa Freitas[1], Ana Rita Gaspar[1], Ana Mara Fonseca[1], Ana Filipa Guedes[1], Luís Mendes Pedro[2], Nuno C. Santos[1], Filomena A. Carvalho[1]

[1] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; [2] Serviço de Cirurgia Vascular, Coração e Vasos, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal.

Email address: catarinalopes@medicina.ulisboa.pt

Background. Carotid artery disease (CAD) is thought to be one of the most common and preventable causes of ischemic strokes. Carotid artery pathologies are associated with atherosclerotic plaque formation on the artery bifurcation, which may present asymptomatically or lead to ischemic stroke or transient ischemic attack1. We have previously reported an increase of erythrocyte adhesion related to blood clots formation and with high plasma fibrinogen levels on different cardiovascular diseases2–5.

Methods. Here, we used atomic force microscopy (AFM) to measure the changes in fibrinogen-erythrocyte and erythrocyte-erythrocyte binding in CAD patients before and after the surgery to remove atherosclerotic plaque. Haemorheological parameters were also assessed. Blood samples were collected from patients before endarterectomy, as well as 6 and 12 months after surgery. The erythrocytes were isolated and washed to removed other blood compounds or medication influence. Results were compared with a control group of healthy blood donors.

Results. CAD patients before surgery, when compared with the control group, had higher erythrocyte stiffness, however it decreased 12 months after surgery. Patients had lower fibrinogen-erythrocyte binding forces after surgery, despite a higher binding frequency. The force necessary to overcome the adhesion between two erythrocytes from CAD patients decreased after surgery in absence of fibrinogen concentration. Haemorheology analysis shows that erythrocytes from CAD patients after surgery have lower propensity to aggregate than before surgery, in agreement with AFM data. High plasma levels of total fibrinogen and □' fibrinogen variant were detected in CAD patients before surgery, decreasing after it.

Conclusions. Changes on erythrocyte stiffness, erythrocyte-erythrocyte adhesion and fibrinogen-erythrocyte binding were observed during CAD patients 12 months follow-up. More research is needed in order to confirm our findings and consider the changes on erythrocyte morphology, fibrinogen interaction, erythrocytes aggregation and adhesion as possible predictive risk factors for CAD. Additionally, the results obtained may contribute for the detection of the possibility of development of restenosis events associated with the carotid artery disease stage.

References.

(1) Fernandes e Fernandes, J.; Mendes Pedro, L.; Gonçalves, I. The Conundrum of Asymptomatic Carotid Stenosis-determinants of Decision and Evidence. *Ann. Transl. Med.* **2020**, *8*, 1279–1279.
(2) Guedes, A. F.; Carvalho, F. A.; Malho, I.; Lousada, N.; Sargento, L.; Santos, N. C. Atomic Force Microscopy as a Tool to Evaluate the Risk of Cardiovascular Diseases in Patients. *Nat. Nanotechnol.* **2016**, *11*, 687–692.
(3) Guedes, A. F.; Moreira, C.; Nogueira, J. B.; Santos, N. C.; Carvalho, F. A. Fibrinogen-Erythrocyte Binding and Hemorheology Measurements in the Assessment of Essential Arterial Hypertension Patients. *Nanoscale* **2019**, *11*, 2757–2766.
(4) Guedes, A. F.; Carvalho, F. A.; Domingues, M. M.; Macrae, F. L.; McPherson, H. R.; Santos, N. C.; Ariëns, R. A. S. Sensing Adhesion Forces between Erythrocytes and γ' Fibrinogen, Modulating Fibrin Clot Architecture and Function. *Nanomedicine* **2018**, *14*, 909–918.
(5) Carvalho, F. A.; Guedes, A. F.; Duval, C.; Macrae, F. L.; Swithenbank, L.; Farrell, D. H.; Ariëns, R. A. S.; Santos, N. C. The95RGD97sequence on the Aα Chain of Fibrinogen Is Essential for Binding to Its Erythrocyte Receptor. *Int. J. Nanomedicine* **2018**, *13*, 1985–1992.

FC8-Vacuolar membrane proteome and role of amino acids in the lifespan of a Niemann-Pick type C1 yeast model

Martins, T. S.[1,2,3], Costa, R. S.[1,2,3], Lemos, C.[1,2,3], Pereira, C.[1,2], Costa, V.[1,2,3]

[1]i3S Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [2]IBMC Instituto de Biologia Celular e Molecular, Universidade do Porto, Porto, Portugal; [3]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Email address: telma.martins@ibmc.up.pt

Background. Lysosome and lysosome-like vacuoles (in yeast) play crucial roles in cellular responses to nutrient availability and composition, ion homeostasis, stress resistance, and cell death. Conserved nutrient signalling pathways, including TORC1, modulate lysosomal function and autophagy. Notably, a decline in lysosomal function has been related with the progression of metabolic and age-associated diseases, including lysosomal storage disorders (LSDs). The Niemann-Pick Type C (NPC) is a rare LSD characterized by an abnormal accumulation of lipids (cholesterol and sphingolipids) in the late endosomal/lysosomal network. It results from loss-of-function point mutations in NPC1 (in 95% of cases) or NPC2, which are involved in lipid transport through the endocytic pathway. We have previously shown that yeast lacking Ncr1p, orthologue of human NPC1 protein, display a premature ageing phenotype associated with mitochondrial dysfunctions and accumulation of sphingolipids [1]. Here, we investigated how vacuolar dysfunction may contribute to *ncr1*Δ shortened lifespan and identity new potential regulators to attenuate mutant phenotypes.

Methods. To characterize the vacuolar membrane proteome, vacuoles were isolated by centrifugation using Ficoll gradients, the vacuolar membranes recovered by ultracentrifugation of osmotically lysed vacuoles and samples analyzed by gel-free mass-spectrometry (LC-MS/MS). To assess chronological lifespan, cells were maintained in growth media over time and viability calculated as the percentage of colony-forming units. TORC1 activity was evaluated by analyzing the levels of phospho-Rps6 (Ser232/233) by western blot. Autophagic flux was estimated through the processing of GFP-Atg8 to GFP.

Results. Our results revealed changes in the levels or phosphorylation of *ncr1*Δ vacuolar membrane proteins that are mostly associated with transmembrane transport (including amino acid transporters), vacuolar acidification, vesicle-mediated transport, and vesicle fusion with vacuole. Consistently, autophagy (process required for amino acid homeostasis) was impaired in *ncr1*Δ mutant and the supplementation of glutamate, glutamine or α-ketoglutarate increased its chronological lifespan and modulated TORC1 activity.

Conclusions. These results suggest that changes in vacuolar proteins and amino acid homeostasis may contribute to the shortened lifespan in the yeast model of Niemann-Pick type C1. This work was funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020. TM (SFRH/BD/136996/2018) and CP (IF/00889/2015) are supported by FCT.

References.

[1] R. Vilaça et al. (2018) Biochim Biophys Acta Mol Basis Dis, 1864, 79-88

S2-PLANT CELL BIOLOGY AND BIOTECHONOLOGY

FC9	Rafael Guerreiro	Assessing drought stress resistance of rice varieties from Guinea-Bissau. I. Functional phenotyping of photosynthetic traits
FC10	Ana Tendeiro	Assessing drought stress resistance of rice varieties from Guinea-Bissau. II. Image-based structural phenotyping
FC11	Mariana Custódio	Characterization of pollen collected in hives located in the region of Samora Correia, Benavente, Portugal.
FC12	Carla Varanda	Development of OMMV as a VIGS vector for plant protection
FC13	Mónica Marques	High Resolution Melting - a promising tool for genotyping walnut plants
FC14	Miguel Silvério	Identification of endophyte microbial communities in long-term in vitro-cultured olive and walnut plantlets and evaluation of isolated olive endophytes in walnut rooting
FC15	Gonçalo Laureano	Lipid signaling events in grapevine response to downy mildew
FC16	Marla Costa & João Carrilho	Macro and micronutrients characterization of two different varieties of grape pomace: Arinto and Touriga Nacional
FC17	Ana Cruz-Silva	Plasmopara viticola effectors gene expression and mycorrhizal grapevine
FC18	Jorge Marques da Silva	Response to drought stress and oligochitosan treatment of Artemisia annua plants
FC19	Teresa Lino-Neto	The potential of cork oak endophyte to be used as biocontrol agent against fungal pathogens
FC21	Ana Alhinho	The dynamics of flower development in sweet chestnut tree (Castanea sativa mill.)

Chairs: Jorge M. Silva (FCUL); Helena Carvalho (CIBIO_UP)

FC9-Assessing drought stress resistance of rice varieties from Guinea-Bissau. I. Functional phenotyping of photosynthetic traits

Rafael Guerreiro[1], Ana Mafalda Tendeiro[1], Luís Catarino[2], Jorge Marques da Silva[1]

[1]BioISI and DBV, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal; [2]CE3C and DBV, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal

Email: fc52656@alunos.fc.ul.pt

Background. One of the greatest challenges we face nowadays is how to sustainably feed the growing human population, in a scenario of extreme climatic phenomena that inflict intense stress upon cultivars. Thereby, it becomes imperative to look for plants more resistant to several stresses, to assure a profitable agriculture and a sustainable use of the earth's resources. The investigation of the phenotypic diversity of local varieties is of extreme importance, since in many countries the population is still dependent on them as the main staple food. Guinea-Bissau is such an example, where the food security of a large portion of the population is dependent on different rice cultivars.

We studied water stress response of two different varieties of Guinea-Bissau's rice, NL-19 (Oryza sativa x Oryza glaberrima) and Masporr (Oryza sativa). Masporr is a local variety, traditionally cultivated in rainfeed upland. NL-19 (Nerica Lowland) is cultivated using a rainfeed lowland cropping system, where it grows in flooded soils. Therefore, we hypothesized that the Masporr variety would be more resistant to drought than NL-19.

Methods. To assess their phenotypic responses, we first germinated the seeds in petri dishes, then transferred to pots with Guinea-Bissau's soil and grown in a phytotron under controlled conditions. During the first 35 days of growth all plants were well-watered and after that half of the plants of each variety were subjected to drought stress by withholding watering for 7 days, while the other half (control) remained irrigated. After that, drought plants were re-watered and monitored for 5 additional days. We periodically measured chlorophyll a fluorescence (continuous (JIP test) and modulated (Rapid Light Curves)), to assess physiological parameters related with the drought response.

Results. The Performance Index was reduced in drought plants as the water stress conditions worsen, more notoriously in NL-19 plants. The photochemical energy fluxes (ABS/RC, effective antenna size; TRo/RC, trapping efficiency; ETo/RC, electron transport; and DIo/RC, dissipation of energy) were less affected by stress in Masporr than in NL-19. The RLC derived parameters (photoinhibition, light saturation, electron transport efficiency and maximum electron transport rate) were only moderately affected in Masporr, but after 5 days of drought treatment variable fluorescence was almost absent in NL-19, making it impossible to compute these parameters and showing a very severe impairment of the photochemical apparatus.

Conclusions. Albeit no recovery was observed after rewatering, Masporr photochemical parameters were less affected by the drought treatment, suggesting that this genotype is more resilient to drought than NL-19, supporting our initial hypothesis.

This work was supported by the FCT Project INTERPHENO (PTDC/ASP-PLA/28726/2017).

FC10-Assessing drought stress resistance of rice varieties from Guinea-Bissau. II. Image-based structural phenotyping

Ana Mafalda Tendeiro[1,2], Rafael Guerreiro[1,2], Miguel Vitorino[2], Margarida Calejo Pires[3], Manuel Abreu[3], Luís Catarino[1,4], Jorge Marques da Silva[1,2]

[1]Departamento de Biologia Vegetal, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal; [2]BioISI, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal; [3]LOLS and Departamento de Física, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal; [4]CE3C, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal, Portugal,

Email address: fc52525@alunos.fc.ul.pt

Background. Considering the increasingly difficult challenge for cropping systems posed by climate change, it is essential to develop methods for selecting new plant varieties resistance to abiotic stresses, such as drought. High throughput plant phenotyping (HTPP) techniques allow to automatically characterize key phenotypic traits in large populations of plants, therefore accelerating the plant breeding processes.

Our study focused on two rice varieties planted in Guinea Bissau, one of them cultivated within lowland conditions, NL-19 (Nerica Lowland (*Oryza sativa* x *O. glaberrima*)) and the other cultivated within upland conditions, Masporr (*O. sativa*).

Methods. Seeds were germinated in petri dishes, then transferred to pots with Guinea-Bissau's soil and grown in a phytotron under controlled conditions. During the first 35 days of growth all plants were well-watered and after that half of the plants of each variety were subjected to drought stress by withholding watering for 7 days, while the other half (control) remained irrigated. After that, drought plants were re-watered and monitored for 5 additional days. At the end of the drought period, varieties were compared regarding their resistance to drought stress, using data generated by a custom-built image-based (RGB and thermographic) plant phenotyping platform (https://interpheno.rd.ciencias.ulisboa.pt/). Traits such as foliar temperature, leaf colour (RGB) and plants average area were measured. Additionally, after rewatering, at the end of the experiment, manually obtained destructive data, such as biomass and leaf area, was collected.

Results. Drought-stressed Masporr plants showed higher leaf temperature than NL-19 drought-stressed plants, suggesting a better stomatal control and a water saving strategy. Also, a stay-green phenotype was observed in Masporr leaves, as confirmed by RGB analysis. Furthermore, at the end of the experiment, Masporr plants showed higher dry mass.

Conclusions. Altogether, these results indicating that Masporr plants were able to save more water, while simultaneously increasing biomass, than NL-19 plants, suggesting that Masporr is the genotype better suited to face drought conditions.

This work was supported by the FCT Project INTERPHENO (PTDC/ASP-PLA/28726/2017).

FC11-Characterization of pollen collected in hives located in the region of Samora Correia, Benavente, Portugal.

Custódio, M.[1], Galveias, A.[2], Raposo, M.[3], Antunes, C. M.[2]

[1] Department of Chemistry, School of Sciences and Technology, University of Évora, 7000-671 Évora, Portugal; [2]Department of Health and Medical Sciences, School of Health and Human Development & ICT—Institute of Earth Sciences, IIFA, University of Évora, 7000-671 Évora, Portugal; [3] Department of Landscape, Environment and Planning, School of Science and Technology, University of Évora, 7000-671 Évora, Portugal; Mediterranean Institute for Agriculture, Environment and Development (MED), University of Évora, 7000-671 Évora, Portugal;

Email address: m47204@alunos.uevora.pt

Background. Beekeeping is a zootechnical practice that is based on the exploitation of colonies of domestic bees *Apis mellifera* and the obtaining of its direct products, e.g, honey and pollen. Currently, bee pollen is a product valued as a dietary supplement, rich in several types of amino acids and vitamins essential to the human body, however, its composition in pollen types is unknown. The main objective of this study was to identify the pollen types found in the bee pollen from hives located in the region of Samora Correia, Portugal.

Methods. Pollen samples were kindly provided by the Beekeeper Mauro Raposo and were collected from the hives during the months of March, April and May 2020 in the region of Samora Correia, Benavente (38°54'18.8"N 8°52'11.1"W). For pollen identification and counting, slides were prepared for observation under an optical microscope at a magnification of 400x. Following literature recommendations, 1200 pollen grains per slide were counted (Louveaux et al., 1978) and classified.

Results. The samples consisted of macroscopic clusters of pollen distinguished by their different colours: yellow, orange, purple, green and brown. Six different pollen types were identified: *Quercus suber, Echium plantagineum, Cistus salviifolius*, genus *Trifolium spp* and species of the families Brassicaceae and Geraniaceae. The most abundant pollen types belonged to the genus *Trifolium spp*, in March (50-65%) and to the species *Echium plantagineum* in April (45-55%) and May (50-60%). An anemophilous species, *Quercus suber*, was also found and constituted 20-30% of the total pollen in March and April and decreased in May (<10%). Different clusters presented a different pollen composition; the orange and purple agglomerates were composed by *Cistus salviifolius* and *Echium plantagineum* pollen, respectively, and was similar throughout the study, while the composition of the remaining clusters varied from month to month.

Conclusions. In summary, the colours of the bee pollen agglomerates is an indicator of composition in specific pollen types. The bee pollen includes pollen from polyniferous (the major component) and nectariferous plants, with a contribution of anemophilous pollen. Bee pollen consists of different pollen types depending on the harvest period. Combining the pollen composition with the knowledge on nutrient composition of the different pollen types may lead to the development of new products with different the nutraceutical properties.

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020.

FC12-Development of OMMV as a VIGS vector for plant protection

Varanda, C.M.R.[1], Félix, M.R. [2], Materatski, P.[1], Campos, M.D.[1], Patanita, M.[1], Marques, N.[3], Clara, M.I.[2], Nolasco, G.[4]

[1]MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [2] MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [3]Departamento de Ciências Biológicas e Bioengenharia da Universidade do Algarve, Laboratório de Virologia, Faro, Portugal; 4 MeditBio, Universidade do Algarve, Campus de Gambelas 8005-139, Faro, Portugal

Email: carlavaranda@uevora.pt

Background. Viruses are responsible for several important plant diseases, however, they have also been used in biotechnology with different purposes. Virus induced gene silencing (VIGS) allows specific silencing of foreign genes that can be inserted in a viral vector and then inoculated in plants. When a sequence of a foreign gene is introduced in a VIGS vector, the plant infected with this vector will be signalized to target that foreign viral RNA and destroy it. In addition, plant defense mechanisms will also target and destroy any homologous RNA, even if it is constitutively expressed by the plant. The VIGS approach can also be used for plant protection purposes; for example, if a fragment of the genome of a pathogenic virus is inserted in a VIGS vector, the plant will destroy it and become protected against a possible further infection of that virus. Several plant viruses have been used as VIGS vectors however, their large genomes, their difficult manipulation and the reduced number of hosts they infect restrain their use as vectors. The Alphanecrovirus Olive mild mosaic virus (OMMV) has characteristics that place it as a very promising vector tool. Its small genome makes it easy to manipulate, and it causes only mild systemic symptoms in a wide range of crops, which will facilitate their manipulation into symptomless constructs and allow its application to a high number of plants.

Methods. An OMMV-based vector is being developed under the project TOMVIRPROTECT. An infectious OMMV full length clone, available at our laboratory (pUC_OMMVFL5), was manipulated to obtain a symptomless OMMV strain. A single mutation at nt18 (C to A) of the OMMV p6, a silencing suppressor protein, formed a STOP codon at this region, resulting in the gene knockout. OMMVp6mutant was then manipulated to carry the GFP reporter gene in its 5' and 3' ends and in both sense and antisense directions to test silencing efficiency.

Results. The new mutated OMMV genome (OMMVp6mutant) was inoculated onto Nicotiana benthamiana indicator plants where it caused no visible symptoms but viral accumulation levels similar to OMMV wild type, as well as a systemic presence. Relative GFP mRNA accumulation level in 16 C plants (plants constitutively expressing GFP) was the lowest when GFP was placed in the 3'end of OMMVp6mutant and in antisense direction. A multiple cloning site was introduced in this region to facilitate introduction of further fragments to be silenced.

Conclusions. These transformations resulted in obtaining an efficient OMMV VIGS vector, which is intended to become available for the control of many important viral plant diseases. This work is funded by the EU through the European Regional Development Fund, under ALENTEJO 2020, ALGARVE 2020 and FCT, under the Projects with references ALT20-03-0145-FEDER-028266 and PTDC/ASPPLA/28266/2017 and ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017.

FC13-High Resolution Melting - a promising tool for genotyping walnut plants

Marques M. [1], João Mota Barroso[2], Julio Nogales-Bueno[3,4] , Rato A.[2] , Cardoso H.[1]*

[1] MED—Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [2]MED & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [3]Food Colour and Quality Laboratory, Área de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Sevilla, 41012, Sevilla, Spain; [4]Department of Animal Production, University of Cordoba, Campus of Rabanales, Córdoba, 14071, Spain.

*Email address: hcardoso@uevora.pt

Background. In recent years, the consumption of walnuts has increased significantly, which consequently have lead to an increase in its global production whith important requirements in terms of marketing standards. In this context, walnut producers have been facing an important problem due to the possibility of mixing fruits from different varieties, that consequently leads to heterogeneous lots with no guaranty of the desired quality. Problems associated to fruits mixing is a relatively frequent situation in commercial orchards of vegetatively propagated plants, and mixtures can occur either in the nurseries due to incorrect identification/ manipulation of plant material used for grafting. Facing this problem, certification of plant material is an important achievement that gives to producers the guaranty of correct identification of plant material for orchards plantation. The methodologies that can assist certification, able to detect problems of frauds/mislabelling are molecular markers known as microsatellites (also known as SSRs – Simple Sequence Repeats). However, this technique is highly cost, require data analysis by a specialized company with a consequent delay in the results and needs technicians with specific training in molecular biology techniques. In an attempt to establish a more easy, faster and reliable method, a methodology based on High Resolution Melting analysis (HRM) is here described.

Methods. Four varieties of walnut tree Junglans regia L. were selected based on it importance at National level - 'Lara', 'Howard', 'Chandler' and 'Tulare'. Plant material, that consisted on leaves, were collected from adult trees established under field conditions. All samples were homogenised with liquid nitrogen and further used for gDNA extraction. Four different methods were tested, three based on commercial kits [Maxwell® 16 Tissue DNA Purification Kit (Promega), Qiagen DNeasy® Plant Mini Kit (Qiagen) and NZY Plant/Fungi gDNA Isolation Kit (NZYtech)], and one based on the CTAB protocol [Doyle and Doyle (1987) Phytochemical Bulletin 19:11-15]. To further proceed with the establishment of the HRM analysis thirteen loci were tested. Parameters like the PCR extension period, annealing temperature, gDNA and primer concentration were considered on the optimization procedure. To get confirmation about the correct identification of varieties, the three loci selected for HRM analysis were also characterized following the SSRs conventional approach [capillary electrophoresis (CE) for fragment size analysis]. Results. Considering the analysis of melting curves it was possible to highlight that the extraction method strongly affected the reliability HRM technique. Three loci were selected to perform discrimination among the four varieties (WGA202, WGA321 and WGA376).

Results achieved indicate that WGA202 locus allows discrimination between var. ‘Tulare’, ‘Lara’ and ‘Chandler’ and both WGA321 and WGA376 loci allow discrimination among the four varieties under study. Fragment analysis by CE revealed fragment size coincident with the variety under study.

Conclusion. The results obtained were promising, allowing the establishment of a promising tool that could be used as an interesting alternative to the common genotyping methods applied in walnut varieties discrimination.

Acknowledgement. This work was funded by National Funds through the Programa Operacional Regional ALENTEJO 2020 under the project QualFastNut (ALT20-03-0246-FEDER-000064).

FC14- Identification of endophyte microbial communities in long-term in vitro-cultured olive and walnut plantlets and evaluation of isolated olive endophytes in walnut rooting

Silvério M.[1], Pires R.[1], Campos C.[1], Peixe A.[2], Cardoso H.[1]*

[1] MED - Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [2]MED & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

*Email address: hcardoso@uevora.pt

Background. Endophytes, microorganisms that colonize internal plant tissues, have been associated with plant growth promotion in *in vitro* tissue culture systems of a diversity of woody plant species. Olive and walnut trees have become important tree crops due to the identification of healthy properties on the olive oil and dry nuts, respectively. For both species, *in vitro* tissue culture systems have been established to massively propagate elite genotypes. However, since the negative influence of some endophytic bacteria have been reported, the identification of the bacterial populations in olive and walnut long-term *in vitro* cultures might help to prevent future problems or allow the identification of plant growth promoting bacteria (PGPB) that can be useful to *in vitro* establish recalcitrant genotypes.

Methods. *In vitro* grown plantlets of olive (*Olea europaea L.*) cv.*'Galega vulgar' cl. 1441* and walnut hybrid 'Paradox' cl. Vlach (*Juglans regia x Juglans hindssi*) were investigated on its endophytic communities' composition. Whole plant DNA was extracted and microbial 16S rDNA was amplified using the primers 779F/1391R (~600bp). After cloning, amplicons were Sanger sequenced and the different taxa were identified, by Blastn at NCBI database. A phylogenetic analysis was also conducted using the mothur toolset at https://usegalaxy.org/. In parallel, leaf segments and steam explants taken from olive and walnut *in vitro* plantlets were cultured in different culture media in order to promote the growth of the endophytes to further investigate its role. *Stenothropomonas rhizophila* isolated from olive was used as potential adventitious root promoter in walnut. Microshoots were inoculated with the bacterial inoculum concomitantly with IBA (rooting inducing hormone) before and during the transition to *ex-vitro* through the following treatments: only IBA (T1), IBA + Bacteria (T2), only Bacteria (T3).

Results. Overall, it was possible to identify members belonging to 9 bacterial families, being some of those olive/walnut-specific endophytes. In the rooting induction experiment performed with *S. rhizophila*, the walnut microshoots from T2 exhibited the better rooting rate, and from T3 the lowest rooting rates. Microshoots from T1 presented smaller and thinner roots in comparison to T2. The T1 and T2 microshoots had similar growth in the aerial part in contrast with microshoots from T3. Microshoots that were inoculated twice with the bacterial inoculum presented similar rooting rates with the corresponding treatment not submitted to the second inoculation.

Conclusions. This work allowed to compare endophytic communities between olive and walnut. The results allowed us to confirm the role of the *S. rhizophila* on promoting adventitious rooting when combined with IBA. Explants exposed to IBA + bacterial inoculum exhibited superior rooting. Walnut cultivars with different behavior upon rhizogenesis stimulus, along with different inoculum concentrations will be considered in future studies in order to improve the applicability of *S. rhizophila* as rooting promoter.

Acknowledgements. This research was funded by National Funds through FCT - Foundation for Science and Technology under the Project UIDB/05183/2020 and by FEDER and National Funds through the ALENTEJO 2020 (ALT20-03-0145-FEDER-000014). The authors are grateful to Virgínia Sobral and Hugo Ribeiro for technical support.

FC15-Lipid signaling events in grapevine response to downy mildew

Laureano G.[1], Cavaco A.R.[1], Matos A.R.[1] Figueiredo A.[1]

[1]Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa, Portugal

Email address: gmlaureano@fc.ul.pt

Background. Viticulture has been highly affected by the downy mildew disease, caused by *Plasmopara viticola*, an obligatory biotrophic oomycete. Understanding the grapevine defense mechanisms is mandatory to develop sustainable disease control measures. Our group has provided strong evidence about the role of fatty acids and lipid signaling in the establishment of the incompatible interaction between grapevine and *Plasmopara viticola*. In response to adversities, changes in membrane lipid composition can alter membrane properties. Moreover, membrane lipids and fatty acids can act themselves as signaling molecules or can be channeled to biosynthetic pathways of other signaling molecules, such as jasmonic acid and other oxylipins, derived from polyunsaturated fatty acids. The generation of polyunsaturated fatty acids results from the activity of fatty acid desaturases, that catalyze the insertion of double bonds into the fatty acids chains of glycerolipids. Despite the importance of lipid modulation in the grapevine defense against downy mildew, the fatty acid desaturase family was not yet characterized and is here presented for the first time.

Methods. The gene family characterization was performed using bioinformatic tools: NCBI databases, MAFT software and RAxML-HPC v.8, on CIPRES Science Gateway. Gene expression analysis of fatty acid desaturases coding genes was done by Quantitative Real Time PCR. Fatty acid profile was performed by Gas Chromatography. Plant material used for gene and lipidomic analyses: *V. vinifera* cv. Regent leaves, mock and inoculated with *Plasmopara viticola*.

Results. The signaling pathways associated to grapevine and *Plasmopara viticola* interaction are still poorly understood, thus we have used a systems biology approach to characterize the genotype-specific differences in grapevine resistance against this pathogen. In the first hours of inoculation with *P. viticola*, Regent, tolerant to downy mildew agent, shows an increase in its unsaturated fatty acids content, mainly in chloroplast lipids. The increase of the unsaturation degree will change the chloroplast membrane properties, becoming more fluid and permeable. Moreover, up-regulation of fatty acid desaturases encoding genes was shown namely those responsible for the unsaturation of chloroplast lipids.

Conclusions. Fatty acid desaturases are key enzymes in the lipid signaling events in the first hours of grapevine-*P. viticola* interaction. These enzymes have a crucial impact in membrane properties, as well as in the production of signaling molecules. Regarding the membrane properties, after interaction with the *Plasmopara viticola*, changes in the lipid and fatty acid composition of chloroplast membranes were observed. The up-regulation of the unsaturation process together with an increase of α-linolenic acid content may be linked to the observed surge in jasmonic acid, an important molecule in grapevine defense towards downy mildew. Our work unveils lipid modulation mechanisms in the establishment of the incompatible interaction between grapevine and *Plasmopara viticola*.

References.

Laureano, G., Figueiredo, J., Cavaco, A. R., Duarte, B., Caçador, I., Malhó, R., Figueiredo, A., “The interplay between membrane lipids and phospholipase A family members in grapevine resistance against Plasmopara viticola,” Scientific Reports, vol. 8, 14538, Sep. 2018.

FC16- Macro and micronutrients characterization of two different varieties of grape pomace: Arinto and Touriga Nacional

Paula Pereira[1,2], Maria Lídia Palma[1], Marla Niza Costa[4], João Carrilho[4], Nuno Saraiva[1], Ana Sofia Fernandes[1], Carla Palma[3], and Marisa Nicolai[1]

[1]CBIOS – Universidade Lusófona's Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749-024 Lisboa, Portugal; [2]CERENA – Center for Natural Resources and Environment Instituto Superior Técnico (IST), Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; [3]Instituto Hidrográfico, R. Trinas 49, 1249-093 Lisboa, Portugal; [4]ULHT – Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal

*Email: marisa.nicolai@ulusofona.pt

Background. Several studies on the chemical and nutritional properties of the by-products of winemaking have been conducted in recent years, showing the various potentials of this product (grape pomace) as a possible ingredient for human consumption, since it has numerous bioactive compounds with high nutritional value and potential health benefits [1,2].

Methods. Two different Portuguese grape pomace varieties were chosen (Arinto and Touriga Nacional) from the Alentejo region. Kjeldahl method was used to determine contents in proteins, Soxhlet extractor to obtain lipid levels, atomic absorption spectrophotometry to analyze the content of metals (Al, Cd, Cr, Cu, Fe, Li, Mn, Ni, Pb, Zn, and Hg), and a hydride generator to analyze the semimetal (As) level. Moisture content was obtained through the mass difference before and after drying in the oven with forced air circulation, ashes was determined by mineralization of the samples at 550º C, and pH by potentiometric method [3]. The crystal violet assay was performed after 24 h of exposure to the ethanolic extracts obtained by ultrasound method to measure human HaCat cells viability [4]. In addition, the percentage of DPPH inhibition was used to predict the antioxidant ability of the ethanolic extracts [3].

Results. The native Portuguese grape varieties Arinto and Touriga Nacional presented protein contents of 8.38±0.07% and 10.13±0.07%, and fat content of 10.29±0.07% and 8.22±0.13%, respectively. The metal with the highest content, in both varieties, was aluminum. Lithium was not detected in Touriga Nacional and in Arinto variety lithium, cadmium, and chromium content were below the limit of detection. The pH values were 4.11±.01 and 4.80±0.01, the ash values were 4.02±0.01% and 5.49±0.01% and the moisture were 3.99±0.01% and 8.88±0.01% for the varieties Arinto and Touriga Nacional, respectively. All the ethanolic extracts led to cell viability levels superior to 90%. The percentages of DPPH inhibition of the ethanolic extracts were around 60%.

Conclusions. The grape pomace samples analyzed presented nutritional interest and antioxidant properties and appear to have a desirable safety profile. These findings can contribute to the rational and evidence-based selection of grape pomace for future inclusion and exploration as a possible human food ingredient.

Funding: This work is funded by national funds through FCT - Foundation for Science and Technology, I.P., under the UIDB/04567/2020 and UIDP/ 04567/2020 project.

References.

1. Garcia-Lomillo, J., García-Lomillo, J., & González-SanJosé, M. L. (2017). Applications of wine pomace in the food industry: Approaches and functions. Comprehensive Reviews in Food Science and Food Safety, 16, 3–22. https://doi.org/10.1111/1541-4337.12238.
2. San'Atnna, V., Christiano, F. D. P., Matczak, L. D. F., Tessaro, I. C., & Thys, R. C. S. (2014). The effect of the incorporation of grape marc powder in fettuccini pasta properties. LWT – Food Science and Technology, 58, 497–501. https://doi.org/10.1016/j.lwt. 2014.04.008 0023-6438.
3. Nicolai, M., Pereira, P., Rijo, P., Amaral, O., Amaral, A., & Palma, L. (2018). Vitis vinifera L. pomace: chemical and nutritional characterization. Biomedical and Biopharmaceutical Research, (15)2, 156-66. doi: 10.19277/BBR.15.2.182
4. Nicolai, M., Almeida, N., Rijo, P., Costa, J.G., Saraiva, N., & Fernandes, A.S. (2019). Cytotoxic effect of antioxidants found in food from plant origin on human osteosarcoma U2OS Cells. Biomedical and Biopharmaceutical Research, (16)1, 89-96. doi:10.19277/bbr.16.1.202

FC17-*Plasmopara viticola* effectors gene expression and mycorrhizal grapevine

Cruz-Silva A[1], Figueiredo A[1] and Sebastiana M[1]

[1]BioISI— Instituto de Biossistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Email address: amcsilva@fc.ul.pt

Background. Grapevine (*Vitis vinifera*), widely used for berry and wine production, is highly susceptible to the pathogenic oomycete *Plasmopara viticola*, the etiological agent of grapevine downy mildew disease. The method commonly used to prevent and control *P. viticola* infection relies on multiple applications of chemical fungicides. However, with European Union goals to lower the usage of such chemicals in viticulture there is a need to develop new and more sustainable strategies. The use of beneficial microorganisms with biocontrol capabilities, such as the arbuscular mycorrhizal fungi (AMF), has been pointed out as a viable alternative. With this study, we intended to investigate the effect of AMF colonization on the expression of *P. viticola* effectors during infection of mycorrhizal grapevine.

Methods. Grapevine plants were inoculated with the AMF *Rhizophagus irregularis* and, after mycorrhizae development, plants were infected with *P. viticola*. The expression of *P. viticola* RxLR effectors was analyzed by real-time PCR during the first hours of interaction.

Results. Results show that pre-mycorrhizal inoculation of grapevine alters the expression of several *P. viticola* effectors; namely, *PvRxLR28*, which presented decreased expression in mycorrhizal plants at the two time points post-infection tested.

Conclusions. These results suggest that the pre-inoculation of grapevine with AMF could interfere with the pathogen's ability to infect grapevine by modulation of pathogenicity effectors expression, supporting the hypothesis that AMF can be used to increase plant resistance to pathogens and promote more sustainable agriculture practices, particularly in viticulture.

FC18-Response to drought stress and oligochitosan treatment of *Artemisia annua* plants

Ana L. García-García[1,2], David Jiménez-Arias[1], Miguel Vitorino[3], Jorge Marques da Silva[3,4]

[1]Instituto de Productos Naturales y Agrobiología - Consejo Superior de Investigaciones Científicas, Spain; [2]Universidad de La Laguna, Spain; [3]BioISI, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal; [4]Departamento de Biologia Vegetal, Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016 Lisboa, Portugal

Email address: jmlsilva@fc.ul.pt

Background. Artemisinin is a sesquiterpene endoperoxide lactone that is used in the treatment of malaria due to its activity against parasites of the genus Plasmodium. It also has activity against several types of cancers, as well as anti-viral and anti-inflammatory properties. Currently, the only commercial source is the plant *Artemisia annua* which produce it in small quantities. As a result of this circumstance, there is a global supply shortfall. Besides, climate change is posing a threat to *A. annua* cultivation. Thus, this requires the optimization of strategies to increase yield. Elicitation is the most prevalent method for increasing a secondary metabolite. This entails the use of either biotic or abiotic elicitors. The first category includes substances derived from fungi, bacteria, viruses, and other plants. The second one can be chemical or physical (such as water stress). Some elicitors are also utilized as biostimulants, such as chitosan, which protects against water stress while also acting as an elicitor in a variety of species, including *A. annua*. Increased yield or increased water resistance may be able to meet the demand for artemisinin. The effects of chitosan treatment and drought stress on artemisinin production as well as physiological changes in *A. annua* plants are presented in this study.

Methods. *A. annua* plantlets were transferred to 1L pots one month after sown and kept one week in a growth chamber. After this time, plants were separated in two groups (well-watered and drought stress) and in each group, three concentrations of oligochitosan were sprayed (0, 100 and 200 $mg \cdot L^{-1}$). Then, water stress was imposed withholding irrigation for 9 days. During these days, plants were monitored with a custom-built image-based phenotyping platform (developed in the INTERPHENO project https://interpheno.rd.ciencias.ulisboa.pt/). At the end of the experiments, leaf samples were taken to artemisinin content, H_2O_2 content, relative water content and water potential.

Results. Drought stress significantly increased artemisinin content at 200 $mg \cdot L^{-1}$ of chitosan, compared to the well-watered plants with the same treatment. Besides, this dose of chitosan improved the relative water content (77.9% ± 6.9) when compared with non-treated stressed plants (44.0% ± 6.6). Thus, under drought stress, water potential was -0.75 MPa ± 0.11 in non-treated plants and -0.35 MPa ± 0.14 in plants treated with 200 $mg \cdot L^{-1}$ chitosan. Regarding H_2O_2 content, drought stress increased it in all groups but this was most evident on the 200 $mg \cdot L^{-1}$ chitosan treatment. Significant differences were also found in the color of plants, assessed by RGB analysis, namely on the median of the green channel, which decreased in chitosan-treated water-stressed plants.

Conclusion. Treating *A. annua* plants with 200 $mg \cdot L^{-1}$ of chitosan may offer the opportunity to cultivate this plant in drought conditions, without loss of yield, since this elicited artemisinin and improve the water status of plants.

Acknowledgements. This work was supported by the FCT Project INTERPHENO (PTDC/ASP-PLA/28726/2017).

FC19-The potential of cork oak endophyte to be used as biocontrol agent against fungal pathogens

Costa, D.[1], Lopes, T.[2], Baptista, P.,[2] and Lino-Neto, T.[1]

[1]BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal, [2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.

Email address: tlneto@bio.uminho.pt

Background. Cork oak is a tree species with high ecologic and socio-economic importance in the Mediterranean region. Cork oak decline caused by microbial pathogens has been increasingly reported and is aggravated by abiotic stressors, such as drought. We hypothesize that a sustainable solution to control disease progression is the use of cork oak endophytic antagonists as biocontrol agents.

Methods. The hypothesis was tested by using a cork oak endophyte (Gr67) in greenhouse assays for controlling charcoal disease (caused by *Biscogniauxia mediterranea*) and bot canker (caused by *Diplodia corticola*). Cork oak seedlings were inoculated with i) negative controls (growing medium); ii) positive controls (Gr67, *B. mediterranea* or *D. corticola*, separately); and iii) pathogen (*B. mediterranea* or *D. corticola*) + Gr67. Fungal spores of Gr67 were inoculated on the trunk of cork oak seedlings pre-exposed to standard irrigation (75% field capacity) and drought conditions (25% field capacity). One week later fungal spores of cork oak pathogens were inoculated on cork oak seedlings. Analysis of several biochemical parameters [chlorophyll *a+b*, phenolics, lipid peroxidation (MDA), hydrogen peroxide content, soluble protein content and peroxidase activity] were evaluated to disclosure defense mechanisms against cork oak pathogens.

Results. Cork oak seedlings inoculated with Gr67 endophyte displayed higher fitness (up to 2-fold for *B. mediterranea* and up to 5-fold for *D. corticola*) at the end of the experiment than those inoculated only with pathogenic isolates. Isolate Gr67 induced defense responses against *D. corticola* by increasing the production of total proteins, MDA and hydrogen peroxide. The mode of action against *B. mediterranea* was not disclosed by these analysis and other defense mechanisms may be involved.

Conclusions. Although more biochemical analyses are needed to understand defense mechanisms, endophytic isolate Gr67 was efficient in conferring protection to cork oak plants against referred pathogens under both water conditions. These findings showed that isolate Gr67 can be considered a potential biocontrol agent and suggested that it will be effective under a climate changing scenario.

FC21-The dynamics of flower development in sweet chestnut tree (Castanea sativa Mill.)

A.T. Alhinho [1], M.J.N. Ramos [2], S. Alves [2], M. Rocheta [2], L. Morais-Cecílio [2], J. Gomes-Laranjo [3], R. Sobral [1], M.M.R. Costa [1]

[1] BioISI- Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; [2] LEAF—Linking Landscape, Environment, Agriculture and Food—Institute of Agronomy, University of Lisbon, Tapada da Ajuda, 1359-017 Lisboa, Portugal; [3] CITAB—Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Universidade of Trás os Montes and Alto Douro, Quinta de Prados, 5000-801 Vila Real

E-mail address: b7682@bio.uminho.pt

Background. The sweet chestnut tree (*Castanea sativa* Mill.) is one of the most significant Mediterranean tree species, being an important resource for wood and fruit. It is a monoecious species, presenting male flowers in staminate catkins and bisexual catkins, with both male and female flowers. Staminate flowers develop in May, whereas the bisexual catkins fully develop one month later. Despite the importance of the chestnut tree, little is known regarding the molecular mechanisms involved in its separate flower development. Thus, the study of *C. sativa* reproductive characteristics is fundamental to fully understand the reproductive success of this species and the impact on its productivity. The development of floral organs has been extensively studied in hermaphrodite species, which led to the proposal of the ABCDE model, in which different classes of MADS-box transcription factors are recruited in the flower meristem to specify the identity of non-reproductive and reproductive organs, in what seems to be a conserved mechanism.

Methods. In the present study, a *de novo* transcriptome for *C. sativa* was assembled and the genes homologous to those of the ABCDE model were identified.

Results. The phylogeny of these genes was inferred, and its expression analysis shows that they are differentially expressed in both unisexual and bisexual catkins. Yeast-two-hybrid analysis suggests that specific changes in the ABCDE interactome could underly the differences necessary to the separate development of male and female flowers.

Conclusions. The MADS-box gene expression and the identification of protein-protein interactions in *Castanea sativa* constitute a step towards the perception of the molecular mechanisms involved in unisexual flower development in this monoecious species.

S3 - TOXICOLOGY AND ENVIRONMENTAL BIOCHEMISTRY

FC22	Ana Galveias	Airborne Quercus spp pollen and adhered particulate matter: physical-chemical characterization in the cities of Évora, Porto and Guarda
FC23	Sander Noordam	Photoelectroautotrophic growth of Rhodopseudomonas palustris TIE-1: The relationship between cytochrome c2 and the reaction center
FC24	Alexandra Penha	Mites in House Dust from Three Cities in Portugal: A Molecular Identification
FC25	António Vieira	Pollen Profiles of House Dust From Three Portuguese Cities With Different Climate Characteristics
FC26	Ana Galveias	Cupressaceae pollen prevalence in Southern Iberian Peninsula: the effect of surface meteorological factors on pollen season intensity
FC27	Adriana Catarino	Ecotoxicological assessment of intermittent streams in the Guadiana Basin
FC28	Ana Cláudia Mendes Gonçalves	Trends of Olea pollen levels between 2009 and 2021: Impact on allergic respiratory disease
FC29	Joana Rodrigues Ferreira Pimpão	Influence of atmospheric pollen levels on the incidence of Sars-Cov-2 virus infection
FC30	Rúben Miguel Dias Duarte	Implementation of a methodology for detecting the SARS-Cov-2 virus um wastewater samples, RT-PCR in Real Time

Chairs: Maria João Bebbiano (UAlg) Patrícia Palma (IPBeja)

FC22-Airborne *Quercus* spp pollen and adhered particulate matter: physical-chemical characterization in the cities of Évora, Porto and Guarda

Galveias, A.[1], Ribeiro, H.[2], Guimarães, F.[3], Costa, M.J.[4], Rodrigues, P.[5], Pereira. J.[6], Costa, A.R.[1], Abreu, I.[6] & Antunes, C.M.[1]

[1] Institute of Earth Sciences (ICT) and Department of Health and Medical Sciences School of Health and Human Development University of Évora, Rua Romão Ramalho, 59, 7000-671 Évora, Portugal M; [2] Institute of Earth Sciences (ICT) and Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, University of Porto, Rua Do Campo Alegre, 687, 4169-007 Porto, Portugal ; [3]Unit of Science and Mineral Technology, National Laboratory of Energy and Geology (LNEG), Portugal; [4] Institute of Earth Sciences (ICT) and Department of Physical, School of Sciences and Technology, University of Évora, Rua Romão Ramalho, 59, 7000-671, Portugal; [5]School of Technology and Management, Polytechnic Institute of Guarda; – Guarda; [6] I. Abreu Institute of Earth Sciences (ICT) and Department of Biology, Faculty of Sciences, University of Porto, Rua Do Campo Alegre, 687, 4169-007 Porto, Portugal

Email address: acgjorge@uevora.pt

Background. The link between the atmospheric particulate matter (PM) and pollen allergy or bronchial asthma is widely documented in the literature, pointing to an adjuvant role of PM in modifying the environment of the respiratory mucosa in which pollen allergens are released, intensifying their response [1]. In fact, when humans breathe, the pollen inhaled enters the nose in a whirlwind manner, which facilitates the PM adhesion to its sticky pollen Wall. Furthermore, the response intensification may be related to the physical characteristics of PM and its toxicity [2]. So, the aim of this study was to characterize the physical-chemical properties of PMs adhered to the airborne pollen, using *Quercus spp* as a model.

Methods: The study was conducted in three Portuguese cities with different urbanization and meteorological conditions (Évora, Guarda and Porto). The sampling was performed during 4 consecutive days (21-24 April in 2017) using a 7-days Hirst-type volumetric sampler, that samples air at 10 L.min-1 simulating the human breathing [3]. The sampled particles impacted on a Melinex tape coated with a double-sided adhesive carbon tape. The Quercus pollen grains were observed using a Field Emission Electron Probe Microanalyser for PM analysis. A secondary electron image was taken of each pollen grain and EDS spectra were obtained for individually adsorbed particles. All images were analysed, and the parameters of the particles adhered in the wall of pollen grains were determined. Back trajectories of air masses arriving at Évora, Guarda and Porto have been calculated using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT) [4,5].

Results. The measurement of the equivalent diameter of the particles ranged from 0-16 □m, however, most particles have a diameter <3µm corresponding to fine particles. For the three cities there were significant differences in the equivalent diameter of the Quercus pollen grains, in the number of particles per pollen, and in the % area occupied by the particles. This indicates distinct Quercus pollen species in the air and Porto had fewer particles per pollen and Évora had a greater number of particles per pollen. Concerning the chemical characteristic, particles of various chemical groups were detected in the three cities, being the Si-rich particles represented in a higher percentage, followed by Ca-rich, Cl-rich, SO-rich particles. In the city of Porto and Guarda organic particles were also found. Remaining chemical species such as SO-rich, P-rich, Metals & Oxides were found in lower concentrations. Particles of Sea Salt were observed in Évora (April 23), coinciding with air mass trajectories from east, possibly carrying these particles from the Mediterranean. Principal component analysis demonstrated a positive association between Si-rich, Ca-rich and SO-rich, P-rich, Metals & Oxides, Ca-rich and Cl-rich levels and relative humidity and negative association with precipitation and wind speed. The maximum, minimum and average temperature have no influence on the particles.

Conclusion: We observed that the physical characteristic of PMs is similar between the studied cities, however, the dominant chemical composition is different, which is an important information regarding their impact in pollen-allergy intensification towards the same pollen type and concentration.

References.

[1] Zhao, C., Wang, Y., Su, Z., Pu, W., Niu, M., Song, S., … Wang, H. (2020). Science of the Total Environment, 730, 139145.
[2] Yoshizaki, K., Brito, J. M., Toledo, A. C., Nakagawa, N. K., Piccin, V. S., Junqueira, M. S., … Macchione, M. (2010). Inhalation Toxicology, 22(7),610–617.
[3] Hirst JM. An automatic volumetric spore trap. Ann Appl Biol. 1952;39(2):257 – 65
[4] Stein, A. F., Draxler, R. R., Rolph, G. D., Stunder, B. J. B., Cohen, M. D., & Ngan, F. (2015). of the American Meteorological Society, 96(12), 2059–2077. https://doi.org/10.1175/BAMS-D-14-00110.1
[5] Rolph, G., Stein, A., & Stunder, B. (2017) Environmental Modelling & Software, 95, 210–228. https://doi.org/10.1016/J.ENVSOFT.2017.06.025

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020 and FCT funded project Pollensorb PTDC/ATPEAM/0817/2014.

FC23-Photoelectroautotrophic growth of *Rhodopseudomonas palustris* TIE-1: The relationship between cytochrome c_2 and the reaction center

Noordam, S.J.[1], Alves, A.S.[1], Trindade, I.B.[1], Madjarov, J.[1] & Louro, R.O.[1]

[1]Inorganic Biochemistry and NMR Laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal

Email address: snoordam@itqb.unl.pt

In order to sustain modern day life while averting the worst consequences of climate change, our society needs to develop industrial processes that are carbon neutral, or even better, carbon negative. Microbial photoelectrosynthesis, during which CO_2 is used as a molecular building block might be a process capable of achieving the latter. With microbial photoelectosynthesis the microorganism captures energy from light to drive the use of electrons collected from a cathode to catalyze the thermodynamically unfavorable reaction of reducing CO_2, to obtain more complex molecules.[1] *Rhodopseudomonas palustris* TIE-1 (R. palustris) is a genetically tractable organism, capable of growing photoelectoautotrophically while using CO_2 as carbon source.[1–3] However, before photoelectrosynthesis by R. palustris can become viable on an industrial scale, further work is essential to optimize and understand this process. Studies indicate that extracellular electron uptake by microbes is the rate limiting step at this moment. Therefore, research efforts have been carried out to improve this, e.g. a novel cathode surface.[3,4] Furthermore, the presence of light proved to significantly influence the ability of R. palustris TIE-1 to perform extracellular electron uptake.[5] The reaction center has been identified as the protein complex responsible for the capture of energy from light.[6] This research is focused on efforts to understand how extracellular electron uptake works. Increasing this understanding might pave the road for effective solutions to increase its efficiency. Previous work indicated that cytochrome c2 is involved in the photosynthetic ability of R. palustris[7], however how and whether this cytochrome interacts directly with the reaction center is not known. Therefore, the light harvesting reaction center complex and cytochrome c2 have been isolated and purified using FPLC. Studying the interaction between cytochrome c2 and the reaction complex is one of the goals of this research project. The investigation of the molecular events that underpin photobioelectrosynthesis, will inform the direction of our research focused on optimizing the performance of *R. palustris* in a 2-chamber bio-reactor that allows it to grow on CO_2 as sole carbon source under photoelectoautotrophic conditions. While growing *R. palustris* under these conditions, we observed that the bacterium still takes up electrons at a potential of 100 mV once the light is turned off, albeit at a very reduced rate. This concurs perfectly with previous studies.[5] Additionally, we observed no soluble extracellular redox active components in the lighted reactor. In this context, the involvement of planktonic cells on extracellular electron uptake will be evaluated. This line of research contributes to the goal of obtaining a working photoelectoautotrophic system with optimized parameters under which *R. palustris* will efficiently produce complex molecules of industrial interest using light, electricity and CO_2.

References

[1] F. W. Larimer, P. Chain, L. Hauser, J. Lamerdin, S. Malfatti, L. Do, M. L. Land, D. A. Pelletier, J. T. Beatty, A. S. Lang, F. R. Tabita, J. L. Gibson, T. E. Hanson, C. Bobst, J. L. Torres Y Torres, C. Peres, F. H. Harrison, J. Gibson, C. S. Harwood, Nat. Biotechnol. 2004, 22, 55–61.
[2] Y. Jiao, A. Kappler, L. R. Croal, D. K. Newman, Appl. Environ. Microbiol. 2005, 71, 4487–4496.
[3] K. Rengasamy, T. Ranaivoarisoa, W. Bai, A. Bose, Nanotechnology 2020, 32, 1–14.
[4] K. Rengasamy, T. Ranaivoarisoa, R. Singh, A. Bose, Bioelectrochemistry 2018, 122, 164–173.
[5] A. Bose, E. J. Gardel, C. Vidoudez, E. A. Parra, P. R. Girguis, Nat. Commun. 2014, 5, 3391–3397.
[6] P. J. Jackson, A. Hitchcock, D. J. K. Swainsbury, P. Qian, E. C. Martin, D. A. Farmer, M. J. Dickman, D. P. Canniffe, C. N. Hunter, Biochim. Biophys. Acta - Bioenerg. 2018, 1859, 119–128.
[7] L. J. Bird, I. H. Saraiva, S. Park, E. O. Calçada, C. A. Salgueiro, W. Nitschke, R. O. Louro, D. K. Newman, J. Bacteriol. 2014, 196, 850–858.

FC24-Mites in House Dust from Three Cities in Portugal: A Molecular Identification

Penha, A.M.[1,6], Vieira, A.[3], Silva, A.[3], Antunes, C.[1,4], Sousa, A.C.A.[5,6], Pastorinho, M.R.[4,6]

[1]Institute of Earth Sciences-ICT, University of Évora, Portugal; [2]Water Laboratory, School of Science and Technology from the University of Évora; [3]Department of Chemistry, University of Évora, Portugal; [4] Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Portugal; [5]Department of Biology, University of Évora, Portugal; [6]Comprehensive Health Research Centre (CHRC), University of Évora, Portugal,

Email address: mapenha@uevora.pt

Background. According to the World Health Organization[1], sensitization (IgE antibodies) to foreign proteins in the environment is present in up to 40% of the world population. In indoor environment, house dust acts as a concentrator of these foreign proteins for which one the most prolific contributors are mites. Modern constructions are very efficient in retaining heat and humidity, the most important factors limiting mites. Moreover, climate change is largely modifying the environment in the same way. This could cause higher concentrations of Der p1, the main allergen associated with the most abundant species in Portugal, *Dermatophagoides pteronyssinus,* and also in Der f1 from *D. farinae* (less abundant due to high sensitivity to low temperature and humidity), but that, given the present changes, could thrive. Furthermore, the current mite distribution map is more than a decade old. Thus, it is important to determine the current mite distribution to create guidelines for the mitigation of allergy in the Portuguese population. In this sense, house dust was collected from 3 cities with different climate profiles: Aveiro (center - littoral); Covilhã (center, interior, altitude); Évora (south, interior, flatland).

Methods. House dust from 5 houses/city was obtained from vacuum bags and deposits after 60 days of use. Samples were sieved (4 meshes) and 4 fractions obtained: 500 – 250 µm; 250 - 125 µm; 125 – 63 µm; and, <63 µm. Isolation of specimens was performed by flotation (5M NaCl). Identification under the microscope using morphological features was impossible due to the fractionation of the recovered specimens. Real-Time Polymerase Chain Reaction (RT-PCR) was carried out after total DNA extraction using TRItidy G™ according to manufacturer instructions. For RT–PCR reaction, each well contained: 1X iTaq™ universal SYBR® Green Supermix (Bio-Rad), 500 nM of each primer sequence, 0.5 µM of Bovine Serum Albumin (BSA) and, 1µL of template DNA, in a total of 20µL per well.

Results. All the different Évora fractioned samples were observed at the optical microscope, but due to the large mixture between pieces of mite and house-dust, it was impossible to identify which species were present, although small pieces of mites could be seen. Therefore, starting from the finest fraction (<63 µm), identification using RT-PCR was performed. In all of those fine fractions from each house/city *D. pteronyssimus* or *D. farinae* mite species were not detected.

Conclusions. The finest fraction (<63 µm) was not the best one to identify which species were present in each house/city. Further analysis using other fractions are going to be performed in order to contribute to update the acarological map of Portugal, published in 2009.

References.

[1]WHO-World Health Organization. White Book on Allergy 2011-2012 Executive Summary. By Prof. Ruby Pawankar, MD, PhD, Prof. Giorgio Walkter Canonica, MD, Prof. Stephen T. Holgate, BSc, MD, DSc, FMed Sci and Prof. Richard F. Lockey, MD.

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020.

FC25-Pollen Profiles of House Dust from Three Portuguese Cities with Different Climate Characteristics

António Vieira[1], Ana Galveias[2], Célia Antunes[2,3], Ana C. Sousa[4,5] & M. Ramiro Pastorinho[3,5]

[1]Department of Chemistry, University of Évora, Portugal; [2]Institute of Earth Sciences-ICT, University of Évora, Portugal; [3]Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Portugal; [4]Department of Biology, University of Évora, Portugal; [5]Comprehensive Health Research Centre (CHRC), University of Évora, Portugal

Email address: antoniovieira.cv@gmail.com

Background. Seasonal allergic rhinitis (commonly known as “hay fever”) is an allergic reaction to pollen from trees, grasses and weeds. This type of rhinitis occurs mainly in the spring and fall, when pollen from trees, grasses and weeds are in the air. However, settled down pollen can concentrate indoors in matrices like house dust, retaining its inflammatory properties, from where it can become airborne again. People with respiratory illnesses like asthma may be more sensitive to pollen, which has been linked to asthma attacks and increases in hospital admissions. In the US alone, medical costs linked with pollen exceed $3 billion. Higher pollen concentrations and longer pollen seasons can also cause sensitization to allergens. Climate change will potentially lead to both these effects, causing more people to suffer more health effects. It is therefore of utmost importance, particularly for allergic patients, to know the predominant species and the concentration of pollen allergens in house dust so as to avoid year-round symptoms, increased costs for the national health service and the spreading of sensitization.

Methods. 15 dust samples were collected in three Portuguese cities (5/city) with different climate profiles: Aveiro (center - littoral); Covilhã (center, inland, altitude); Évora (south, inland, flatland). Samples were obtained from vacuum cleaner bags and deposits after a 60-day period of use and sieved through a 63 µm mesh. Pollen was separated by sucrose gradient centrifugation (60g/L).. Pollen counts were performed under the optical microscope and pollen spectra were determined by counting 100 pollen grains per slide.

Results. In Évora and Covilhã the dominant species was *Quercus* spp, with 70% and 60% of all pollens counted, followed by Poaceae (11% and 12%, respectively). The third place in Évora was occupied by *Pinus* spp. (5%) and in Covilhã by *Olea* spp. In Aveiro, Poaceae pollen was the most abundant with 42% of counts followed by *Quercus* spp. (23%) and *Pinus* spp. (8%). In Évora the indoor pollen spectra resembles the outdoor.

Conclusions. Pollen spectra in house dust was different between cities and is closely related to the relative abundance of outdoor pollen. This is most probably a result of the plant distribution at the different regions, with oak and pine trees dominating the landscape in inland territory while being scarce in the littoral areas. O the other hand, the period of sample collection might explain why olive pollen is within the most representative in Aveiro (samples collected in May/June) but not in Évora and Covilhã (samples collected in March/April), before the olive season in the latter. Future studies are necessary to establish the risk of exposure to allergenic pollen indoor.

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020.

FC26-Cupressaceae pollen prevalence in Southern Iberian Peninsula: the effect of surface meteorological factors on pollen season intensity

Ana Galveias[1], Maria João Costa[2], Paloma Cariñanos[3,4], Consuelo Diaz de La Guardia[4], Ana Rodrigues Costa[1] & Juan Luis Guerrero-Rascado[3,5], Célia M. Antunes[1]

[1]Institute of Earth Sciences - ICT & Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal; [2]Institute of Earth Sciences - ICT & Department of Physics, School of Sciences and Technology, University of Évora, Évora, Portugal; [3]Andalusian Institute for Earth System Research (IISTA-CEAMA), University of Granada, Granada, Spain; [4] Botany Department, Sciences Faculty, University of Granada, Granada, Spain; [5] Applied Physics, Department Sciences Faculty, University of Granada, Granada, Spain.

Email address: acgjorge@uevora.pt

Background: The *Cupressaceae* family is considered a significant source of airborne allergen and allergy and its pollen is reported worldwide. Due to its use as ornamental trees in many cities of the Iberian Peninsula, *Cupressaceae* pollen is a great source of urban allergies in late-winter and early-spring [1]. The objective of this study was to analyse the aerobiology of *Cupressaceae* pollen and the influence of meteorological factors prior to pollination in two sites with environmental differences: Évora (Portugal) and Granada (Spain).

Methods: Data were collected in two cities from southern Iberian Peninsula, namely Évora (38.5685°N; -7.9105°W; 240 m asl) and Granada (37.1041°N; 3.3555°W; 680 m asl), characterized by rural/fat and urban/mountains environmental, respectively. The Cypress is a symbolic tree for both cities but for the city of Granada there are 4000-5000 trees distributed in its neighbourhoods, contributing to the very high pollen exposure. The *Cupressaceae* pollen was monitored using standard Hirst-type traps (2017-2019) and identified by optical microscopy, according to the standard methodology [2]. The surface meteorological variables were obtained from ICT/CGE platform (Portugal) and Agencia Estatal de Meteorología (AEMET, Spain). According to the Köppen climate classification Évora and Granada present typical Mediterranean climate (Csa), characterized by a temperate climate with warm and dry summer. The seasonal Tmax is similar between sites (~18ºC), but the Tmin and Tmean are lower in Granada, ~5ºC and ~2ºC, respectively. The rainfall period, occurring mostly between autumn and spring, presented an average annual precipitation of 608.5 and 352 mm, in Évora and Granada, respectively [3,4].

Results: Large concentrations of pollen were detected in both cities, 5-6-fold higher in Granada. The Seasonal Pollen Integral, SPI, ranging from 3053-7824 pollen /m3 in Évora and 16654-57968 pollen/m3 in Granada. The peak date occurred between 25th February and 14th March in both cities and the pollen season duration (PSD) was similar, ranging from 72-157 days; nevertheless, Granada presented twice as many days with high risk for allergic outbreaks (>100 pollen/m3). Winter Tmax (November-January) and early autumn precipitation and relative humidity (RH) (September-November) positively correlated with SPI. PSD was particularly affected by precipitation and RH during the season, with longer seasons correlating with higher precipitation and RH.

Conclusions: Meteorological factors, particularly, the autumn rain and the temperature during winter months are relevant for *Cupressaceae* SPI and might constitute indicators for the prediction of pollen seasonal intensity, hence, to improve the allergy risk forecast models.

References

[1]. Shahali, Y., Poncet, P., & Sénéchal, H. (2013). Pollinose aux Cupressacées et pollution atmosphérique. Revue Francaise d'Allergologie, 53(5), 468–472. https://doi.org/10.1016/j.reval.2013.01.050;
[2] REA. (n.d.). Retrieved July 23, 2021, from https://www.uco.es/investiga/grupos/rea/;
[3] Instituto Português do Mar e da Atmosfera, from http://www.ipma.pt/pt/index.html/;
[4] Agencia Estatal de Meteorología - AEMET. Gobierno de España. (n.d.);

Acknowledgments. This work was co-funded by the European Union through the European Regional Development Fund, framed in COMPETE 2020 (Operational Programme Competitiveness and Internationalization) and national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020, and FCT funded projects Pollensorb-PTDC/ATPEAM/0817/2014 and NanoSen-SOE2/P1/E0569.

FC27-Ecotoxicological assessment of intermittent streams in the Guadiana Basin

Catarino A[1]; Martins I. [1]; Fialho S. [1]; Lima A.[1]; Palma, P.[1,2]
[1]Department of Technologies and Applied Sciences, Polytechnic Institute of Beja 7801-295, Portugal; [2]ICT, Institute of Earth Sciences, University of Évora, Rua Romão Ramalho 59, Évora, Portugal

Email address: adri.catarino@hotmail.com

Background. Degradation of ecosystems at different spatial and temporal scales occurs through multiple stressors, resulting in a loss of biodiversity and in an impoverishment of ecosystem services. This situation becomes much more worrying in regions where the water scarcity is a serious problem, as in Mediterranean. In fact, the Mediterranean regions have been considered as the most exposed to the impacts of climate change, with increasing average temperatures and reduced annual precipitation. This reality is more problematic in intermittent streams, more influenced by the seasonality and intensification of extreme events. The efficient management of surface waters depends on the development and selection of sensitive and easy-to-apply tools allowing to rank the pressures, for further mitigation of their effects. The objective of this work was the ecotoxicological assessment of Guadiana Basin streams.

Methods. Samples were collected in four streams of Guadiana Basin (Álamos, Amieira, Lucefécit and Zebro) in six different periods of 2017 (January, March, May, July, September and November) and bioassays were carried out with organisms of different trophic levels: (i) the bacteria *Vibrio fischeri;* (ii) the microalgae *Pseudokirchneriella subcapitata*; and (iii) the crustaceans *Daphnia magna* and *Thamnocephalus platyurus*.
Results. The ecotoxicological results showed sublethal effects (growth inhibition; see Figure 1) induced mainly with the samples from Zebro and Lucefécit streams. In short-term bioassays *T. platyurus* was most sensitive than *V. fischeri* and the Zebro and Amieira were the streams that presented water samples more toxics (*V. fisheri*: 30 min-EC_{50} % $_{(Zebro)}$ = 8.92 ± 0.10; EC_{50} % $_{(Amieira)}$ = 40.77 ± 2.42; and *T. platyurus*: 24h - EC_{50} % $_{(Zebro)}$ = 65.84 ± 2.42; EC_{50} % $_{(Amieira)}$ = 81.36 ± 14.92). January is the month with higher ecotoxicological risk.

Conclusions. Thus, the ecotoxicological results indicated that some of the streams analysed may be suffering a strong impact on their aquatic communities, with the corresponding imbalance in their ecosystem services. This reality was more evident in the wet period, when the pressure of climatic conditions was more evident.

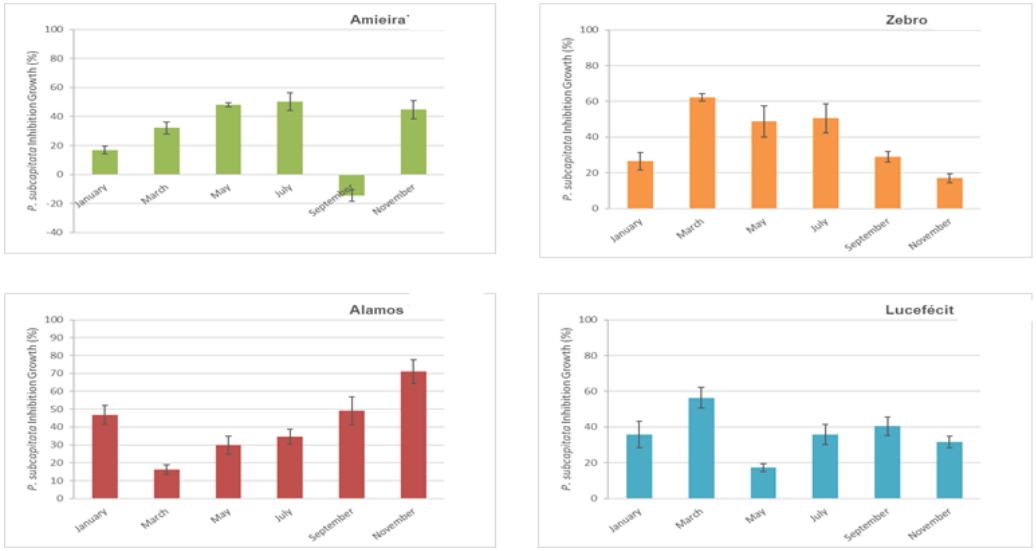


Figure 1 - P. subcapitata growth inhibition (%) in streams of Guadiana Basin.

Funding. The present presentation is supported by the European Union through the European Regional Development Fund, included in the COMPETE 2020 (Operational Program Competitiveness and Internationalization) through the ICT project (UIDB/04683/2020) with the reference POCI-01-0145-FEDER-007690 and by the ALOP project (ALT20-03-0145-FEDER-000004), and through the AquaQ2 project (ALT20-03-0145-FEDER-039494/SAICT-ALT/39494/2018).

FC28-Trends of *Olea* pollen levels between 2009 and 2021: Impact on allergic respiratory disease

Gonçalves A.[1], Galveias A.[2], Alpizar-Jara R.[3,4], Costa M.J. [2,5], Antunes C.M.[2,6] & Costa A.R.[2,6]

[1]Department of Chemistry, School of Science and Technology, University of Évora, Portugal; [2]Institute of Earth Sciences - ICT; [3]Research Center in Mathematics and Applications – CIMA; [4]Department of Mathematics, School of Sciences and Technology, University of Évora, Portugal; [5]Department of Physics, School of Sciences and Technology, University of Évora, Portugal;[6]Department of Health and Medical Sciences, School of Health and Human Development, University of Évora, Portugal.

E-mail address: l43189@alunos.uevora.pt

Background. The olive tree (*Olea europaea*) is a fruit tree with great longevity, which pollination occurs during the spring months [1]. In the region of Évora this pollen type is very abundant due to the high number of specimens existing in the region. About 40% of pollen allergic individuals in the Évora region are sensitized to this pollen type [2]. Therefore, monitoring the evolution of atmospheric levels of olive pollen is very relevant. This work aimed to study the evolution of olive pollen levels between 2009 and 2021, in the Évora region, and evaluate their impact on allergic respiratory disease.

Methods. The pollen was collected using a HIRST-type sampler, placed in an elevated area in the city and was later identified and counted, according to the standard method [3]. Daily pollen concentration was expressed as number of pollen grains per m^3 of air. Meteorological data were obtained from the Atmospheric Sciences Observatory station (ICT). Data on sales of antihistamine drugs were provided by the Center for Health Studies and Assessment (CEFAR).

Results. The Annual pollen Index (API) varied between 2043 and 15314 pollen/m^3 in the study period, corresponding to the years 2020 and 2021, respectively. The years with a higher number of days with high allergic risk (>200 pollen grains/m^3) were 2009, 2011 and 2021 (with 20, 20 and 22 high risk days, respectively). In the period analysed (2009-2021) no significant API trend was detected, despite the increasing area of olive groves. The precipitation during the pollen season favoured its duration but does not significantly affect the API. Additionally, a greater number of rainy days seems to favour the occurrence of more low-risk days during the pollinic season (r=0,6208, p=0.074397). In the period studied, the year with the highest sales volume of anti-allergic drugs, 2009, corresponds to a year in which olive tree pollen was very intense, addressing an association between olive pollen and allergic symptomatology.

Conclusions. The results suggest that olive tree pollen in the Évora region contributes to the worsening of allergy symptoms in the population. For its allergic relevance, it will be necessary in the future to continue to monitor the olive pollen in the region, and to continue to investigate which parameters contribute to its air concentration and prevalence.

References.

[1] *Oliveira | Brigada da Floresta*. (n.d.). Retrieved July 2, 2021, from https://brigadadafloresta.abae.pt/oliveira/
[2] *Polen Alert*. Retrieved July 1, 2021, from https://lince.di.uevora.pt/polen/index.jsp
[3] Galán Soldevilla, C. Spanish Aerobiology Network (REA): Management and Quality Manual; Servicio de Publicaciones, Universidad de Córdoba: Córdoba, Spain, 2007; ISBN 9788469063538.

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020.

FC29-Influence of atmospheric pollen levels on the incidence of Sars-Cov-2 virus infection

Joana Pimpão[1], Ana Galveias[2], Russell Alpizar-Jara[3,4], Ana R. Costa[2,5], Célia M. Antunes[2,5]

[1]Department of Chemistry, School of Science and Technology, University of Évora, Portugal; [2]Institute of Earth Sciences - ICT; [3]Research Center in Mathematics and Applications – CIMA; [4]Department of Mathematics, School of Sciences and Technology, University of Évora, Portugal; [5]Department of Health and Medical Sciences, School of Health and Human Development, University of Évora, Portugal.

Email address:l42606@alunos.uevora.pt

Background. Nowadays, despite the pandemic situation, all the factors that influence the spread and transmission of the SARS-CoV-2 virus or its infective capacity are not yet known [1]. This study aims to analyze the influence of airborne pollen levels on the transmission of the Sars-Cov-2 virus in the regions of Évora and Lisboa and Vale do Tejo (L&VT).

Methods. For the present study, the number of daily incidence of SARS-Cov-2 infections and pollen concentration (pollen/m^3) during in 14 months of pandemic were monitored. Pollen was collected with a Hirst-type volumetric trap [2,3]. Daily Sars-Cov-2 infections data were collected from an Esri-Portugal database according to the official bulletins of the General Directorate of Health and to Évora was from the database of the City Council of Évora. The temporal distribution was analyzed, and the periods of confinement identified. An autocorrelation and a partial correlation analysis were performed to the period with the highest incidence of SARS-COV-2, in the absence of confinement (between October 6, 2020 and February 14, 2021).

Results. The results show that the variation profiles of both pollen and the Sars-Cov-2 infection incidence are very similar in the two different regions. The pollen levels recorded in L&VT were lower than Évora (15±18 and 23±37 pollen/m^3/day, respectively, in the study period) while the number of daily new infections in L&VT were higher than in Évora (1918±1673 and 23±19 daily infectious cases, respectively). For Évora, a mild negative (-0.218) but significant (p = 0.015) correlation between pollen concentration and the logarithm of the new cases of Sars-Cov-2 was determined. When a three-day moving average of both data sets are applied and excluding days with pollen ≥ 120 pollen grains/m^3, a mild positive (0.322) but highly significant (p=0.000) correlation was obtained. These results suggest that the days with very high pollen can be decisive for the relation between the two variables. It should be noted that in the L&VT region daily pollen concentration was always below 120 pollen/m^3, thus it was not possible to establish any significant correlation.

Conclusions. More studies will be needed to better understand the relation between pollen concentrations and the Sars-Cov-2 infection incidence. The relation between aerosolized agents with inflammatory and/or infectious potential may provide a better understanding of the interaction mechanisms of these agents on the immune system, and to the development of forecasting models and risk assessment tools.

References:

[1] A. Damialis, S. Gilles, M. Sofiev, V. Sofieva, and F. Kolek, “Higher airborne pollen concentrations correlated with increased SARS-CoV-2 infection rates , as evidenced from 31 countries across the globe,” vol. 118, no. 12, pp. 1–10, 2021, doi: 10.1073/pnas.2019034118/-/DCSupplemental.Published;

[2] Hirst, B. Y. J. M. (1945). C 257 1. *Assessment*;

[3] “MANUAL DE CALIDAD Y GESTION DE LA RED ESPAÑOLA DE AEROBIOLOGIA, REA - Free Download PDF.” https://silo.tips/download/manual-de-calidad-y-gestion-de-la-red-espaola-de-aerobiologia-rea (accessed Jul. 24, 2021).

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020

FC30-Implementation of a methodology for detecting the SARS-CoV-2 virus um wastewater samples, RT-PCR

Duarte. R.[1], Morais. M[2,3,4] Penha. A.M.[2,4]

[1]Department of Chemistry, University of Évora, Portugal; [2]Institute of Earth Sciences-ICT, University of Évora, Portugal; [3]Department of Biology, University of Évora, Portugal; [4]Water Laboratory, School of Science and Technology from the University of Évora

Email address: rubendduarte3@gmail.com

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 in Wuhan, Hubei province, China, and rapidly spread globally, being considered as a Pandemic, by the World Health Organization (WHO) on March 11, 2020. SARS-CoV-2 ribonucleic acid (RNA) has been detected in the stool of not only symptomatic but also asymptomatic patients [1, 2, 3, 4, 5, 6, 7]. These clinical observations suggest that municipal wastewater from affected communities may contain the virus. Wastewater-based epidemiology is a promising approach to understand the prevalence of viruses in a given Wastewater Treatment Plant (WWTP) watershed because wastewater contains viruses excreted from symptomatic and asymptomatic individuals in a watershed [8, 9]. This epidemiological method is especially useful for early warning of disease outbreaks and informs the effectiveness of public health interventions, as demonstrated above for enteric viruses such as norovirus, hepatitis A virus, and poliovirus. The abstract presented here represents a future study and preliminary results for the methodology implementation.

Therefore, this study focuses on the implementation of a methodology for concentrating and detecting the SARS-CoV-2 virus in wastewater samples using Real-Time Polymerase Chain Reaction (RT-PCR).

Methods. Wastewater samples will be collected from different WWTP (entry and exit samples), using a 24-hour automatic sampler, in different Alentejo cities (Évora, Portalegre, and Elvas) and underlying hospitals. The methods used to carry out this study are Tangential Flow Filtration which serves so that the collected samples are properly filtered so that they are more concentrated and clearer so that the concentration of any virus that is present therein, be as much as possible. Total RNA extraction from wastewater samples will be performed using the E.Z.N.A.® Total RNA kit II, Omega, Bio-Tek (USA) according to manufactures instructions. For SARS-CoV-2 RT-PCR detection it will be used the ViroReal® kit SARS-CoV-2 Multiplex.

Results. Preliminary results showed that the Tangential Flow Filtration method is suitable to concentrate total RNA from wastewater samples, although it is a time-consuming technique.

Conclusions. The complete methodology from concentration methodology and SARS-CoV-2 RT-PCR identification will be analysed.

References:

1. Gao, Q.Y., Chen, Y.X., Fang, J.Y., 2020. 2019 novel coronavirus infection andgastrointestinal tract. J. Dig. Dis. 1–2.
2. Holshue, M., DeBolt, C., Lindquist, S., Lofy, K., Wiesman, J., Bruce, H., Spitters, C., Ericson,K., Wilkerson, S., Tural, A., Diaz, G., Cohn, A., Fox, L., Patel, A., Gerber, S., Kim, L.,Tong, S., Lu, X., Lindstrom, S., Pallansch, M., Weldon, W., Biggs, H., Uyeki, T., Pillai,S., 2020. First case of 2019 novel coronavirus in the United States. N. Engl. J. Med.382, 929–936.
3. Jiehao, C., Jing, X., Daojiong, L., Lei, X., Zhenghai, Q., Yuehua, Z., Hua, Z., Xiangshi, W.,Yanling, G., Aimei, X., He, T., Hailing, C., Chuning, W., Jingjing, L., Jianshe, W., Mei, Z.,Children, N., Women, H., Central, S., Zeng, M., 2020. A case series of children with 2019 novel coronavirus infection: clinical and epidemiological features. Clin. Infect. Dis.
4. Tang, A., Tong, Z., Wang, H., Dai, Y., Li, K., Liu, J., Wu, W., Yuan, C., Yu, M., Li, P., Yan, J., 2020. Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China. Emerg. Infect. Dis. J. 26.
5. Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Mueller, M.A., Niemeyer, D., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Bruenink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., Wendtner, C., 2020. Virological assessment of hospitalized cases of coronavirus disease 2019. Nature. https://doi.org/10.1038/s41586-020-2196-x
6. Zhang, J., Wang, S., Xue, Y., 2020. Fecal specimen diagnosis 2019 Novel Coronavirus–Infected Pneumonia. J. Med. Virol. 0–2. https://doi.org/10.1002/jmv.25742
7. Zhang, W., Du, R.H., Li, B., Zheng, X.S., Yang, X. Lou, Hu, B., Wang, Y.Y., Xiao, G.F., Yan, B., Shi, Z.L., Zhou, P., 2020. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg. Microbes Infect. 9, 386–389.
8. Sinclair, R.G., Choi, C.Y., Riley, M.R., Gerba, C.P., 2008. Pathogen surveillance through monitoring of sewer systems. Adv. Appl. Microbiol. 65, 249–269.
9. Xagoraraki, I., O'Brien, E., 2020. Wastewater-based epidemiology for early detection of viral outbreaks, in: O'Bannon, D. (Ed.), Women in Water Quality. Springer Nature Switzerland, pp. 75–97. https://doi.org/10.1007/978-3-030-17819-2.

S4 - STRUCTURAL BIOLOGY AND MOLECULAR MODELLING / S7 – MEMBRANES AND CELL BIOPHYSICS

FC31	Mickael Diallo	Post-translational modification of STAT3 modulates its cellular distribution
FC32	Andreia Silva	Gold compounds' inhibitory effect on Aquaporin-3 impairs melanoma cell migration
FC33	Ana Manuela Gonçalves	Interaction of MT-SC22EK peptide, HIV fusion inhibitor with membrane models
FC34	Jorge Martins	On the water solubility of fatty acids: interplay of molecular structure and pKa
FC35	Maria Berrocal Carrillo	The potency of gold (I and III) compounds as PMCA inhibitors and their effects on cell viability
FC36	Catarina Gonçalves Pimpão	Unraveling the mechanism of aquaporin-10 irreversible inhibition by organogold compounds
FC37	Anjos L. Macedo	Preliminary Structural characterization of human Gla Rich Protein involved in the inhibition of mineral crystal formation in vascular calcification
FC38	Toni Rendulic	Unravelling the Structural Features of Saccharomyces cerevisiae Acetate Transporter Ady2/Ato1
FC39	Nelly Silva	Unravelling dengue virus capsid protein interaction with viral genome
FC40	Cátia Santos-Pereira	Integrated biochemical and computational approach reveals novel mechanisms underlying the antifungal activity of the milk-derived lactoferrin protein

Chairs: Cláudio Soares (ITQB NOVA); Graça Soveral (FFUL); Nuno Santos (FMUL)

FC31-Post-translational modification of STAT3 modulates its cellular distribution

Diallo M.[1,2,3], Letra-Vilela, R.[1,2,3], Martins-Alves, D.[1,2], Herrera, F.[1,2,3]

[1]Cellular structure and dynamics laboratory, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal.; [2]Instituto de Biossistemas e Ciências Integrativas (BioISI), Faculdade de Ciências, Universidade de Lisboa, Portugal. [3]MOSTMICRO Research Unit, Instituto de Tecnologia Química e Biológica (ITQB-NOVA), Universidade Nova de Lisboa, Oeiras, Portugal

Email address: mdiallo@fc.ul.pt

Background. Signal Transducer and Activator of Transcription 3 (STAT3) is a ubiquitous and pleiotropic transcription factor involved in major physiological and pathological processes such as normal development, immunity, and tumorigenesis. Its cellular localization determines its functions, notably at nuclear or mitochondrial levels. Moreover, STAT3 activity is regulated by molecular interactions and post-translational modifications (PTMs). While more than 80 residues are susceptible to PTMs, a little number of them has been widely studied. We aim at identifying relevant PTMs involved in STAT3 cellular localization and function, with a focus on lesser studied residues.

Methods. Our group developed a Venus-STAT3 bimolecular fluorescence complementation (BiFC) assay that allows the visualization and study of protein-protein interactions in living cells, including STAT3 homo- and heterodimerization. Residues susceptible for PTMs were blocked by site-directed mutagenesis on STAT3-Venus BiFC constructs. To rule out the interference of endogenous STAT3, we produced STAT3-Knockout (STAT3-KO) cell lines via CRISPR/Cas9 methodology. Cellular localization of STAT3 dimers was visualized by wide-field and confocal fluorescence microscopy.

Results. STAT3-KO cells lacked expression of STAT3 at the protein level. STAT3-KO cell lines transfected with wild-type STAT3-Venus BiFC constructs mainly showed diffuse cytoplasmic and nuclear signal of STAT3 homodimers. Additionally, perinuclear aggregates are visualized in a fraction of transfected cells. Transfection with K49R or Y68F BiFC pairs led to a reduction in the percentage of cells with perinuclear aggregates. Cytokine-stimulation led to a rapid nuclear translocation (within 10 min.) at the expense of cytoplasmic STAT3 dimers, leaving small foci of STAT3 dimers anchored at the plasma membrane. This effect was abrogated by the Y705F mutation, which prevents STAT3 phosphorylation.

Conclusions. We showed that mutations at specific N- or C-terminal residues may affect the localization of STAT3 dimers in non-stimulated STAT3-KO cells. We are currently screening all putative phosphorylatable residues for their effects on the intracellular distribution and function of STAT3 dimers in living cells. The outcomes will provide a strong basis for drug targeting STAT3 in the context of a wide spectrum of physiological and pathological conditions regulated by this transcription factor.

FC32-Gold compounds' inhibitory effect on Aquaporin-3 impairs melanoma cell migration

Silva A.G.[1,2], da Silva I.V.[1,2], Casini A.[3], Soveral G.[1,2]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, [2]Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, [3] Department of Chemistry, Technical University of Munich, München, Germany

Email address:silvaandreiagoncalves@gmail.com

Background. Skin covers the entire human body surface, being its first line of defense. Aquaporins (AQPs) are channels that facilitate water and small neutral solutes movements across membranes, being key proteins with implication in skin physiology, such as hydration, cell proliferation and differentiation and wound healing [1], as well as in skin disease, such as skin eczema and melanoma. The involvement of AQP3 in oncogenesis, underlined it as a promising target in the development of novel skin cancer therapeutics [2]. Previously, the gold compound Auphen was revealed as a potent inhibitor of AQP3 activity[3]. Here, we aimed to unveil new potent AQP3 inhibitors to investigate the role of AQP3 in tumor signaling pathways and tumorigenesis.

Methods. First, we evaluated the effect of new potent AQP3 inhibitors on cell migration of melanoma cells. For that, a new series of gold compounds resultant from Auphen modifications (RBA29, $C^{NH}N$, $C^{CO}N$, $C^{CH2}N$, STAM013, RBA31), were tested for the toxicity and ability to block cell migration of melanoma MNT-1 cell line, with high expression of AQP3. The cytotoxicity of the compounds was determined by MTT assay at different concentrations to find the maximal concentration where at least 70% of cells were viable after 24h of incubation. To investigate the effect of AQP3 inhibition in cell migration, the wound closure assay was performed in melanoma cells incubated without or with gold compounds at 5μM.

Results. Our results showed that most of compounds, below 5μM, proved to be harmless or induced less than 20% loss of cell viability. RBA31 and RBA29 were highly cytotoxic, causing high loss of viability. All compounds delayed melanoma cell migration compared to the control condition where the wound was totally closed in less than 24h. $C^{CO}N$ exhibited the strongest inhibitory effect on cell migration (60%), followed by $C^{NH}N$ (53%), $C^{CH2}N$ (26%) and STAM013 (22%).

Conclusions: Altogether our data reveal AQP3 as a key player in cell migration and cancer growth, unveiling its potential as a targetable molecule against tumors where AQP3 is highly expressed, thus, shedding light on novel therapies for treating melanoma.

References.

1. Patel R, Kevin Heard L, Chen X, Bollag WB. Aquaporins in the Skin. Advances in experimental medicine and biology. 2017, 969, 173-191, doi:10.1007/978-94-024-1057-0_11.
2. da Silva IV, Silva AG, Pimpão C, Soveral G. Skin aquaporins as druggable targets: Promoting health by addressing the disease. Biochimie. 2021 Sep; 188:35-44. doi: 10.1016/j.biochi.2021.05.019. Epub 2021 Jun 12. PMID: 34097985.
3. Martins AP, Marrone A, Ciancetta A, Galán Cobo A, Echevarría M, Moura TF, Re N, Casini A, Soveral G. Targeting aquaporin function: potent inhibition of aquaglyceroporin-3 by a gold-based compound. PLoS One. 2012, 7(5), e37435. doi: 10.1371/journal.pone.0037435.

FC33-Interaction of MT-SC22EK peptide, HIV fusion inhibitor with membrane models

Gonçalves A[1]., Martins do Canto A[1].

[1]Universidade de Évora - Escola de Ciências e Tecnologia, Portugal

Email address: l31127@alunos.uevora.pt

Background. HIV interacts with target cells through the action of the viral glycoproteins gp41 and gp120. Over time, the HIV virus has undergone several mutations that lead to the development of resistance to drugs that inhibit virus fusion [Shi et al, 2016]. One of the fusion inhibitors to suffer this resistance was Enfuvirtide (T-20), one of the first fusion inhibitors to be approved by the FDA (Food and Drug Agency) [Oishi et al., 2010]. Thus there is a continuing need to develop new inhibitors to effectively fight the disease. The way in which certain fusion-inhibiting peptides interact with membranes in solution and the maintenance of stable helical structures has been correlated with an increase in the inhibitory capacity of HIV and target cells. Thus, in the development of this work, it was observed by molecular dynamics (MD) the interaction of the MT-SC22EK peptide, an HIV fusion inhibitor, with POPC and POPC:Chol (1:1) membrane models and the effect of the group MT compared to SC22EK peptide, based on results obtained by Pronto [Pronto, 2019].

Methods. To this end, the MT-SC22EK peptide was constructed based on its amino acid residue sequence, with the Arguslab 4.01 program [Thompson, 2004] and the initial membrane models were built with the aid of a toolkit from the GROMACS simulation package 2016 [Abraham et al, 2015; Bekker et al., 1993; Berendsen et al., 1995; Hess et al., 2008; Lindahl et al., 2001; van der Spoel et al., 2005; Páll et al., 2015; Pronk et al., 2013;]. Then, parameters were calculated to evaluate the behavior of the peptide such as the distance from the center of mass of the peptide to the surface of the membranes, the position of the Cα, secondary structures of the peptide, the Lennard-Jones and Coulomb energies in the final systems, the diffusion and the rotational dynamics of the peptide. To evaluate the behavior of the membranes, the parameters were calculated, with and without adsorbed peptide, the membrane area and thickness, the lateral diffusion coefficients of the lipids, the order parameters and the rotational dynamics of certain molecular axes and the angles of important axes.

Results. The results obtained show that, once the peptide is adsorbed, the bilayers present different behaviors and undergo changes in their structure and diffusion, as well as the peptide itself. The MT-SC22EK peptide showed greater efficacy compared to the SC22EK peptide.

Conclusions. Based on all the results obtained throughout the work, it is concluded that the MT-SC22EK peptide has good characteristics and properties as an HIV virus fusion inhibitor, as it has a strong binding with the POPC:Chol membrane and maintains its helical structure, it was also observed that the MT group influences the antiviral activity of the peptide under study.

FC34-On the water solubility of fatty acids: interplay of molecular structure and pK_a

Jorge Martins[1,2]

[1]CCMAR, Faro, Portugal, 2DCBB-FCT, Campus de Gambelas, Universidade do Algarve, Faro, Portugal

Email address: jmatin@ualg.pt

Background. Fatty acids are ubiquitous molecules in biological systems. Both free and forming complex lipids, they play a number of biochemical roles: major metabolic fuel (storage and transport of energy), essential building blocks of all biomembranes, and signaling (eicosanoids and regulation of gene transcription). In addition, they are employed in diverse industrial uses: *e.g.* food, pharmaceutical and personal hygiene industries. Hence, the characterization of the interactions of free fatty acids dispersed in water and its thermodynamics basis is of crucial importance in several areas of research and development. Yet, reports on the aqueous solubility of fatty acids are scarce, timeworn and inconsistent [Bell, 1973; Vorum *et al.*, 1992]. This work presents a systematic and reliable evaluation of water solubility of fatty acids at 25 □C, as well as their correspondent pK_a values. It is well known that fatty acids are poorly soluble in water in their undissociated form, thus the actual solubility, particularly of longer-chain acids, is often very difficult to determine since it is markedly dependent on both pH and fatty acyl chain length. Structure-based methods to estimate water solubilities overcome these difficulties, offering speed and upmost accuracy for most practical purposes. Whenever available from literature, the estimated values of water solubilities and pK_a, will be compared with tabulated values [PubChem], for the sake of completeness and critical assessment.

Methods. The estimations of water solubility values at 25 □C are carried out through an atom contribution approach [Hou *et al.*, 2004] and their pH dependency, using the corresponding calculations with the Henderson-Hasselbälch equation [Shoghi *et al.*, 2013]. This work is only concerned with water solubility at pH=2 (unionized fatty acids) and at pH=7 (mostly ionized). The estimate of pK_a values are obtained through calculations of partial charge distribution of atoms in molecules [Csizmadia *et al.*, 1997].

Results. It is worth to highlight that the solubility limit of amphiphilic molecules, such as fatty acids, corresponds to the formation of supramolecular aggregate in aqueous media, for instance: monolayers, micelles or bilayers, depending on the acyl length and on the presence of other ions and temperature. The change of a water solution to an aqueous suspension of aggregates is known as CAC (critical aggregation concentration). This way, the solubility at pH=7 and 25 □C of common saturated fatty acids varies from 2.88□$10^{□□}$ M for lauric acid (C12), until 2.4□$10^{□□}$ M for arachidic acid (C20). The values of pK_a are independent of the acyl chain length, being consistently 4.95. Arachidic acid is in practice insoluble in water at pH=7 and 25 □C, which poses some pertinent questions about its aggregation state in biological systems or in handling of industrial aqueous formulations.

Conclusions. Introductory calculations for the common saturated fatty acids show that water solubility (S) at 25 □C is about 2 orders of magnitude lower at pH=2 than at pH=7. This is an expected outcome, since at pH=2 all the fatty acids are in the unionized state. Furthermore, the plots of log S *vs.* number of carbons in acyl chain (n_C) are linear, both unionized and mostly ionized at pH=7, and the thermodynamics basis of this practical relationship is derived within the framework solubility equilibrium equations.

Acknowledgments. This study received Portuguese national funds from FCT - Foundation for Science and Technology through project UIDB/04326/2020.

FC35-The potency of gold (I and III) compounds as PMCA inhibitors and their effects on cell viability

Berrocal, M. [1], Cordoba-Granados, J.J.[1], Carabineiro, S.A.C. [2], Aureliano, M. [3,4] and Mata, A.M. [1]*

[1]Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, and Instituto de Biomarcadores de Patologías Moleculares, Universidad de Extremadura, 06006 Badajoz, Spain
[2]LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; [3]FCT, Universidade do Algarve, 8005-139 Faro, Portugal; [4]CCMar, FCT, Universidade do Algarve, 8005-139 Faro, Portugal

*Correspondence: anam@unex.es

Background. Plasma membrane calcium ATPases (PMCA) are key proteins in the maintenance of calcium homeostasis. Dysregulation of PMCA function has been associated with several human pathologies, including neurodegenerative diseases and therefore these proteins are potential drug targets to counteract those diseases. Gold compounds, namely of Au(I), are well-known for their therapeutic use in rheumatoid arthritis and other diseases for centuries. Herein, we report for the first time the ability of four Au(I and III) compounds to interefere with PMCA activity.

Methods. Au(I) and Au(III) complexes were used. Dichloro(2-pyridinecarboxylate)gold(III) (**1**) was purchased from Aldrich. The Au(I) complexes chlorotrimethylphosphinegold(I) (**2**), 1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene gold(I) chloride (**3**) and chlorotriphenylphosphine gold(I) (**4**) were purchased from Strem Chemicals. Steady-state assays of the PMCA activity were measured spectrophotometrically at 37 ˚C, using the coupled enzyme pyruvate kinase/lactate dehydrogenase assay, as described elsewhere [1]. Purified pig brain PMCA was reconstituted in phosphatidylcholine IIS and added to the assay medium. Activities were measured after subsequent additions of 1 mM ATP and freshly prepared gold solutions. All experiments were performed at least in triplicate. The inhibitory power of the tested gold compounds was evaluated by determining their half maximal inhibitory concentration (IC_{50}). SH-SY5Y neuroblastoma cell cultures and treatments were performed as described in [3].

Results. The Au(III) compound (**1**) inhibits PMCA activity with and IC_{50} values of 4,9 μM, while Au(I) compounds (**2**, **3** and **4**) inhibit the protein activity with IC_{50} values of 2.9 μM, 18.1 μM and 0.9 μM, respectively. Regarding the native substrate MgATP, gold compounds **1** and **4** showed a non-competitive inhibition, whereas compounds **2** and **3** showed a mixed inhibition. The results suggested that different compounds have different modes of interaction with the PMCA, and both Au(III) as Au(I) compounds showed good inhibitory capacity for this enzyme. Gold compounds, at the assayed concentrations are shown to be cytotoxic to SH-SY5Y human neuroblastoma cell cultures. However, co-treatment of cells with methylene blue partially prevented cell death.

Conclusions. PMCA activity is inhibited by both Au (I and III) compounds. Their IC_{50} values of inhibition are in the range of 0.9 to 18 μM. Values particularly important were found for the Au(I) compound **4** (IC_{50} < 1 μM), similar to the ones previously described recently for gold compounds **2** and **4** with SERCA (sarco and (endo) reticulum Ca^{2+}-ATPase) [2]. The results obtained with compound **4** with and IC_{50} < 1 μM show that this Ca^{2+}-ATPase can be used as a therapeutic target in medicinal inorganic chemistry. Gold compounds affect SH-SY5Y cells viability.

References.

[1] Salvador, J.; Mata, A.M. *Biochem. J*, **1996,** *315*, 183-187
[2] Fonseca, C.; Fraqueza G.; Carabineiro, S.A.C.; Aureliano, M. *Inorganics*, **2020**, *8*, 49.
[3] Berrocal *et al., Int. J. Mol. Sci.,* **2019**, 20, 3521

Funding. This research was funded by grants from the Spanish Ministerio de Economia y Competitividad (BFU2017-85723-P) to AM and from Fundação para a Ciência e a Tecnologia (FCT) projects UIDB/04326/2020 (MA), UIDB/50006/2020 and UIDP/50006/2020 (LAQV) and Scientific Employment Stimulus—Institutional Call (CEECINST/00102/2018).

FC36-Unraveling the mechanism of aquaporin-10 irreversible inhibition by organogold compounds

Pimpão.C.[1,2], Wragg. D.[3], Bonsignore.R.[3], Aikman. B.[3,4], Pedersen. P. A.[5], Leoni. S.[4], Casini. A.[3] and Soveral. G.[1,2]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; [2]Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; [3]Department of Chemistry, Technical University of Munich, 85747 Garching bei München, Germany; [4]School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, United Kingdom; [5]Department of Biology, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen OE, Denmark

Email address: pimpaocatarina@gmail.com

Background. Aquaporins (AQPs) are membrane protein channels that facilitate the diffusion of water and small solutes across cell membranes. AQPs have emerged as promising drug targets for their involvement in a variety of pathologies, unveiling the use of potent and selective AQP modulators as promising approaches for treatment of AQP-associated diseases. Organometallic gold(III) complexes have gained interest with Auphen being discovered as a potent human AQP3 inhibitor [1]. The mechanism behind this interaction was also reported, showing a reversible binding between the gold metal and AQP3 Cys40 residue [2]. Here, we investigated the inhibitory effect of new cyclometalated Au(III) C^N compounds in human AQP10 (hAQP10), an aquaglyceroporin expressed in the adipose tissue with relevance in body energy homeostasis [3].

Methods. Using aqy-null yeast cells transformed with a plasmid encoding hAQP10, we tested the compounds' inhibitory effect on glycerol permeability using stopped-flow fluorescence. Knowing that these compounds can react with cysteine residues and lead to the formation of stable and irreversible C-S bonds, we evaluated the reversibility of organogold compounds bond to hAQP10 cysteine residues, in the presence of β-mercaptoethanol. Moreover, we investigated their binding mechanism through molecular modelling and metadynamics atomistic simulations.

Results. Permeability assays revealed Au(III) $C^{CO}N$ complex as one of the most potent of the cyclometalated Au(III) C^N compound series to inhibit hAQP10-mediated glycerol transport. These compounds revealed to irreversibly inhibit hAQP10-mediated glycerol permeability, probably due to the establishment of C-S bonds. Computational studies revealed a local arylation of hAQP10 Cys209 residue by Au(III) $C^{CO}N$ complex, resulting in alteration of the glycerol conductance pathway with overall shrinkage of the pore, while water flux was barely affected. Thereby, even if the arylation occurs at a distance from the channel selectivity filters, the whole pore responds to this local modification.

Conclusions. Altogether, we found Au(III) $C^{CO}N$ complex as a potent inhibitor of hAQP10 glycerol permeability and identified a new mechanism of hAQP10 irreversible modulation by establishment of a C-S bond.

References

1. Martins, A.P., et al., Targeting aquaporin function: potent inhibition of aquaglyceroporin-3 by a gold-based compound. PLoS One, 2012. 7(5): p. e37435.
2. Serna, A., et al., Functional inhibition of aquaporin-3 with a gold-based compound induces blockage of cell proliferation. J Cell Physiol, 2014. 229(11): p. 1787-801.
3. Gotfryd, K., et al., Human adipose glycerol flux is regulated by a pH gate in AQP10. Nat Commun, 2018. 9(1): p. 4749.

FC37-Preliminary Structural characterization of human Gla Rich Protein involved in the inhibition of mineral crystal formation in vascular calcification

Macedo. AL[1], Silva. JP[1], Ângelo. R[1], Viegas.CSB[2,3], Simes.DC[2,3]

[1]UCIBIO - Applied Molecular Biosciences Unit, Departamento de Química, and Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology | FCT-UNL, Caparica, Portugal, [2]Centre of Marine Sciences, University of Algarve, Portugal; [3]GenoGla Diagnostics, CCMAR, University of Algarve, Portugal

Email address: Anjos.Macedo@fct.unl.pt

Background. Vascular calcification (VC) is recognized as an active, highly controlled, cell-mediated process of osteochondrogenic differentiation of vascular smooth muscle cells (VSMCs), sharing many features with bone development. Human Gla-rich protein, hGRP, is a vitamin K-dependent protein, with high content in carboxyglutamic acid (Gla) residues, 15 in a total of 74. Gla residues are known for their ability to bind metals, specifically calcium ions. Furthermore, GRP can also bind calcium crystals such as hydroxyapatite, which can be found in mineralized extracellular matrix. We have recently shown that hGRP acts as an inhibitor of VC and its function is probably associated with preventing calcium-induced signalling pathways and direct mineral-binding to inhibit crystal formation/maturation.

To understand the molecular mechanism involved in this inhibition, we explore the structural features of hGRP by NMR spectroscopy, one of the main techniques in protein structure determination, allowing to explore protein-ligand interactions in solution and in confined media.

Methods. Recombinant hGRP, rhGRP, was expressed in *E. coli* BL21 (DE3) cells and isotopically labeled with ^{15}N and ^{13}C. Solubilization and refolding of the protein from inclusion bodies was optimized during the purification process. Non-□-carboxilated protein was prepare in phosphate buffer for NMR experiments: i) ^{1}H-^{15}N HSQC, ^{13}C detected CON and ^{13}C-^{13}C-FLOPSY experiments were collected in a Bruker 600 MHz AVANCE III spectrometer; ii) HR-MAS experiments were performed at 500MHz, in a lyophilized sample of rhGRP, obtained after overnight incubation with commercial hydroxyapatite.

Results. The ^{1}H-^{15}N HSQC spectrum of rh-GRP reveals poor chemical shift dispersion of the resonances, characteristic of an unfolded, or partially folded protein. ^{13}C-detected experiments are used to achieve a better resonance dispersion as observed in ^{13}C-CON experiments. Several Thr and Ser spin systems can be identified in a preliminary assignment of ^{13}C-^{13}C-FLOPSY spectrum. The preliminary HR-MAS experiments confirmed that rh-GRP can also bind to hydroxyapatite and that we can use this system to characterize this protein-ligand interaction.

Conclusions. This is the first structural study of rhGRP using solution NMR. NMR experiments showed that rhGRP is an intrinsically disordered protein and ^{13}C detected experiments are the most efficient way of assigning the protein's residues due to the increase in spectral resolution.

References.

Viegas CSB, et al. (2018) "Chronic Kidney Disease CCPs and EVs Promote VC: A Role for GRP" *Arterioscler. Thromb. Vasc. Biol.*, 38, 575–87

Viegas CSB *et al.* (2009) "Gla-rich protein is a novel vitamin K-dependent protein present in serum that accumulates at sites of pathological calcifications," *Am. J. Pathol.*, 175, 2288–98

Acknowledgments – see presentation

FC38-Unravelling the Structural Features of *Saccharomyces cerevisiae* Acetate Transporter Ady2/Ato1

Rendulic T, Alves J, Soares-Silva I, Casal M
Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal

Email address: toni.rendulic@bio.uminho.pt

Organic acids are ubiquitous compounds in the nature while also being of high industrial relevance, with application in polymer, food, agricultural and pharmaceutical sectors. Plasma membrane transporters are typically required for cells to consume organic acids as carbon sources or to export them as metabolic products, since organic acids predominantly exist in their negatively charged form when the pH is higher than their pK_a value, and therefore cannot cross the plasma membrane via simple diffusion. Recently, membrane transporters started receiving greater attention in metabolic engineering strategies for microbial production of organic acids. *Saccharomyces cerevisiae* is a common microbial platform for organic acid production because of its tolerance to acidic conditions and simplicity of its genetic manipulation. Ato1 is the main transporter responsible for the uptake of acetic acid into the cytosol in *S. cerevisiae* (1). It is also able to mediate organic acid transport in the opposite direction, as it was shown to be involved in the export of lactic acid from *S. cerevisiae* cells engineered for lactic acid production (2). Ato1 is a member of the Acetate Uptake Transporter Family (AceTR) (3), with several functionally characterized homologues in yeast, fungi, and bacteria (3, 4, 5). The recently solved crystal structure of its bacterial homologue, SatP, depicts a hexameric anion channel. In this work, we studied the relationship between structure and function of Ato1 via rational mutagenesis approach. After physiologically characterizing *S. cerevisiae* cells expressing mutated *ATO1* alleles, we identified residues critical for Ato1 substrate specificity and transport activity. By utilizing computer-assisted three-dimensional modelling tools, we provide possible explanations of acquired phenotypes. Our final goal is to test the applicability of these transporters in yeast cell factories to improve the production of organic acids.

References

1. Paiva, S., Devaux, F., Barbosa, S., Jacq, C., Casal, M., 2004. Ady2p is essential for the acetate permease activity in the yeast *Saccharomyces cerevisiae*. Yeast 21: 201–210
2. Pacheco, A., Talaia, G., Sá-Pessoa, J., Bessa, D., Gonçalves M.J., Moreira, R., Paiva, S., Casal, M., Queirós, O., 2012. Lactic acid production in *Saccharomyces cerevisiae* is modulated by expression of the monocarboxylate transporters Jen1 and Ady2. FEMS Yeast Research 12(3): 375-81.
3. Ribas, D., Soares-Silva, I., Vieira, D., Sousa-Silva, M., Sá-Pessoa, J., Azevedo-Silva, J., Viegas, C., Arraiano, M.C., Diallinas, G., Paiva, S., Soares, P., Casal, M., 2019. The acetate uptake transporter family motif “NPAPLGL(M/S)” is essential for substrate uptake. Fungal Genetics and Biology, 122: 1-10
4. Sá-Pessoa, J., Amillis, S., Casal, M., Diallinas, G. 2015. Expression and specificity profile of the major acetate transporter AcpA in *Aspergillus nidulans*. Fungal Genetics and Biology 76: 93–103.
5. Sá-Pessoa, J., Paiva, S., Ribas, D., Silva, I. J., Viegas, S. C., Arraiano, C., Casal, M. 2013. SatP (YaaH), a succinate-acetate transporter protein in *Escherichia coli*. Biochemical Journal 454: 585-95.

FC39-Unravelling dengue virus capsid protein interaction with viral genome

Nelly M. Silva[1], Ana S. Martins[1], Nina Karguth[1], Francisco J. Enguita[1], Roland G. Huber[2], Nuno C. Santos[1], Ivo C. Martins[1]

[1]IInstituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649- 028 Lisbon, Portugal; [2]Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), 138671 Singapore, Singapore

Email address: nellysilva@medicina.ulisboa.pt

Background. Dengue virus (DENV) and Zika virus (ZIKV) are mosquito-borne flaviviruses, sharing structural features. The nucleocapsid core of the mature virion is formed by the 11 kb viral (+) singlestranded RNA condensed with multiple copies of the capsid (C) protein. This is an essential protein, conserved among flaviviruses, which is involved in key steps of the viral life cycle, namely encapsidation and viral assembly. One key step, essential for viral replication, requires DENV C specific binding to intracellular lipid droplets (LD), an interaction that was fully characterized. In addition to the interaction with LDs, it was also demonstrated by us that DENV C interacts specifically with host very low-density lipoproteins (VLDL) and viral RNA. Those findings led to the development of pep14-23, a patented peptide based on the region comprising amino acids 14 to 23 of DENV C, which is able to inhibit DENV C binding to LDs and VLDL.

Methods. Then, in order to characterize DENV and ZIKV C binding to the viral RNA, through biophysical approaches, we started examining locations within the viral RNA to which the protein has higher affinity, with specific RNA sequences being identified.

Results. Preliminary circular dichroism data show that some analogous DNA sequences used as proxies of selected RNA sequences do indeed interact with DENV C, causing changes in the protein secondary structure.

Conclusions. Other biophysical approaches, such as dynamic light scattering, fluorescence and nuclear magnetic resonance spectroscopies, are being applied to better characterize this phenomenon, DENV C regions to which the viral RNA is prone to bind. These data will allow to select, test and develop inhibitors against this essential interaction of DENV C with viral RNA. This methodology used for DENV might be applied to other related flaviviruses (such as ZIKV), as well as other human viral pathogens.

FC40-Integrated biochemical and computational approach reveals novel mechanisms underlying the antifungal activity of the milk-derived lactoferrin protein

Cátia Santos-Pereira[1, 2], María T. Andrés[3], Juliana F. Rocha[4], Henrique S. Fernandes[4], Susana R. Chaves[1], José F. Fierro[3], Hernâni Gerós[1], Stéphan Manon[5], Sérgio F. Sousa[4], Lígia R. Rodrigues, Manuela Côrte-Real[1*]

[1]Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, 47101- 057 Braga, Portugal, [2]Centre of Biological Engineering (CEB), Department of Biological Engineering, University of Minho, 47102-057 Braga, Portugal, [3]Laboratory of Oral Microbiology, University Clinic of Dentistry (CLUO), and Department of Functional Biology (Microbiology), Faculty of Medicine, University of Oviedo, 33006 Oviedo, Asturias, Spain, [4]UCIBIO@REQUIMTE, BioSIM, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Porto, Portugal,[5] Institut de Biochimie et de Génétique Cellulaires, UMR5095, CNRS et Université de Bordeaux, CS61390, 1 Rue Camille Saint-Saëns, 33000 Bordeaux, France

Email address: catia91pereira@gmail.com

Background. Lactoferrin (Lf) is a naturally occurring iron-binding protein and one of the most well-known milk bioactive compounds. It exhibits a remarkable wide-spectrum antifungal activity against a wide collection of yeasts and filamentous fungi. However, the molecular mechanisms underlying this activity are still poorly elucidated, which limits Lf application as an antifungal agent. To deepen our understanding on this subject, the role of plasma membrane ergosterol- and sphingolipid-rich lipid rafts and their association with the proton pump Pma1p, previously identified as a Lf-binding protein, was explored. The effect of Lf on the vacuolar proton pump V-ATPase was also addressed.

Methods. A combination of biochemical and computational studies was developed to unravel the mechanisms underlying Lf's antifungal activity.

Results. We showed that bovine Lf (bLf) perturbs ergosterol-rich lipid rafts organization by inducing intracellular accumulation of ergosterol. Furthermore, we found that perturbations in the composition of these membrane domains increase resistance to bLf-induced yeast cell death, and that when Pma1p-lipid rafts association is compromised, the bLf killing activity is impaired. bLf further inhibited the activity of V-ATPase leading to vacuolar alkalinization. By employing a multi-level computational approach, we unraveled the putative mechanism by which Lf inhibits V-ATPase and identified key binding residues that will certainly aid in the rational design of follow-up experimental studies, bridging in this way computational and experimental biochemistry.

Conclusions. We believe our data will help to pave the way for the use of bLf for the treatment/eradication of clinically and agronomically relevant yeast/fungal pathogens

S1/S5 - SPN-MOLECULAR MECHANISMS OF DISEASE/NEUROBIOLOGY OF AGING AND STRESS

FC41	Samantha Mancino	Allele-specific transcriptional and epigenetic signatures of the aging brain
FC42	Joana Poejo	Amyloid β(1-42) binding to calmodulin and lipid rafts inhibits L-type calcium channels in cerebellar granule neurons
FC43	Fernanda Murtinheira	Ataxia related protein sacsin knockout disrupts the intermediate filaments network in glial cells
FC44	Beatriz Martins	Inflammation induces retinal pigment epithelium monolayer disruption and release of distinct populations of extracellular vesicles
FC45	Leonor Cancela	Involvement of MGP in African Spiny mice ear regeneration
FC46	Sunil Poudel	MEK/ERK and cAMP/PKA mediate HIF-1α signalling during mitochondrial antioxidant induced osteoblastogenesis
FC47	Susana Costa	Pursuing the key residues behind Dis3L2 specificity
FC48	Daniela Martins Alves	The role of understudied post-translational modifications on STAT3 behavior and function
FC49	Ana Ledo	Hydrogen Peroxide as an Intercellular Redox Signaling Molecule in the Brain: Diffusivity, Uptake and Removal
FC50	Maria Teixeira	Autosomal recessive spastic ataxia of Charlevoix-Saguenay: A 3D epidermal model to understand sacsin function in the skin
FC51	Pedro Corda	Proteomic analysis as a tool to identify key differentially expressed proteins during human sperm cryopreservation
FC52	Débora Varela	Involvement of Znf687 during osteoblast proliferation and differentiation

Chairs: Tiago Oliveira (UMin), João Laranjinha (UC)

FC41-Allele-specific transcriptional and epigenetic signatures of the aging brain

Mancino S [1,2,3], Mupo A [4], Krueger F [5], Eckersley-Maslin M [4,6], da Rocha ST[1,2,3]

[1]Instituto de Medicina Molecular, João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal; [2]Departamento de Bioengenharia e Instituto de Bioengenharia e Biociências, Instituto Superior Técnico, Universidade de Lisboa, Portugal; [3] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Portugal; [4]Epigenetics Programme, Babraham Institute, Cambridge CB22 3AT, UK; [5]Bioinformatics Group, Babraham Institute, Cambridge CB22 3AT, UK; 6 Peter MacCallum Cancer Centre, Melbourne, Victoria 3000, Australia.

Email address: samantha.mancino@medicina.ulisboa.pt

Background. Aging -specific epigenetic drift has been mostly studied at the level of DNA methylation, where consistent changes in 5-methycytosine levels at several genomic regions have been noticed. DNA methylation is a common mechanism regulating monoallelic expression of different genes, including imprinted genes expressed in a parent-of-origin-specific manner. How age-related alterations of methylation levels varies across alleles and impact on monoallelic expression is not known. In the brain, this phenomenon may contribute mechanistically to neuronal dysfunction and diseases. In order to investigate the allele-specific transcriptional and epigenetic signatures of aging, we used key brain areas, hippocampus (HCP) and cerebellum (CB), of juvenile and old hybrid mice obtained from BL6×CAST/EiJ (CAST) reciprocal crosses.

We set out to explore whether epigenetic drift affects global DNA methylation machinery by investigating 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) levels.

Methods. Mass spectrometry analysis showed a significant increase of 5hmC levels in HCP of old mice. This was associated with a decrease of the expression of ten eleven translocation methylcytosine dioxygenase (Tet) enzymes. The expression of several repetitive elements (LINEs, SINEs, IAP) was also analysed, strictly dependent on 5mC and 5hmC levels, and reporting a reduction of the intracisternal A-particle (IAP) retrotransposons in HCP of old mice.

Results. We then uncovered the allelic-specific DNA methylation profile of aging using genomic imprinting as a read-out by taking advantage of IMPLICON, a targeted amplicon sequencing method for imprinted regions with unprecedented resolution. Our results showed imprinting does not seem substantially affected during aging and did not affect their normal monoallelic expression for the loci analyzed.

Conclusions. This work provides the first epigenetic and transcriptional landscape of aging with allelic resolution. This knowledge will be valuable to find novel biomarkers of aging that might be helpful in the early diagnosis of neurodegenerative diseases.

FC42-Amyloid β(1-42) binding to calmodulin and lipid rafts inhibits L-type calcium channels in cerebellar granule neurons

Poejo, J[1], Salazar, J[1,2], Mata, AM[1,3] and Gutierrez-Merino, C[1,3]

[1]Instituto de Biomarcadores de Patologías Moleculares, Universidad de Extremadura, 06006 Badajoz, Spain;
[2]Departamento de Química, Universidad Nacional Autónoma de Nicaragua-León, León 21000, Nicaragua;
[3]Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, 06006 Badajoz. Spain.

Email address: joanapoejo86@gmail.com

Background. Lipid rafts are a primary target in studies of amyloid β (Aβ) cytotoxicity in neurons because these plasma membrane submicrodomains have been shown to bind exogenous Aβ peptides and also play a major role in their uptake by neurons leading to the formation of strongly neurotoxic intracellular Aβ aggregates. On the other hand, dysregulation of intracellular calcium homeostasis in neurons has been observed in both sporadic and familial forms of Alzheimer's disease (AD). Besides, several calmodulin (CaM) binding proteins particularly relevant in the control of neuronal cytosolic calcium homeostasis and calcium signaling are associated with lipid rafts, like L-type calcium channels, Ca^{2+}/CaM-dependent protein kinase II and plasma membrane calcium pumps. In a previous work we have shown that Aβ(1-42), the prevalent Aβ peptide found in the amyloid plaques of AD patients, bind with high affinity to purified CaM (dissociation constant ≈ 1nM [1]).

Methods. In this work, to experimentally assess the Aβ(1-42) binding capacity by intracellular CaM, a calcium-buffering protein highly expressed in neurons, we have used primary cultures of mature cerebellar granule neurons (CGN) as a neuronal model. The main methodological approaches used in this work are co-immunoprecipitation, Western blotting, fluorescence microscopy imaging (including fluorescence resonance energy transfer) and cytosolic calcium measurements.

Results. Our results show a large complexation of submicromolar concentrations of Aβ(1-42) dimers by CaM in CGN, up to 120 ±13 picomoles of Aβ(1-42) /2.5 x 10^6 cells. Using fluorescence microscopy imaging we show an extensive co-localization of CaM and Aβ(1-42) within the same lipid rafts in CGN stained with up to 100 picomoles of Aβ(1-42)-HiLyte^{TM}-Fluor555 monomers. In addition, we found that the resting cytosolic calcium of mature CGN in partially depolarizing 25 mM potassium medium is largely lowered by exposure to Aβ(1-42) dimers during 2 hours, conditions that produced an internalization of less than 100 picomoles of Aβ(1-42) dimers in neuronal somas.

Conclusions. We found that in these experimental conditions the primary cause of this decrease of the resting cytosolic calcium is the inhibition of L-type calcium channels of CGN by Aβ(1-42) dimers.

References.

[1] Corbacho, I.; Berrocal, M.; Török, K.; Mata, A.M.; Gutierrez-Merino, C. High affinity binding of amyloid β-peptide to calmodulin: Structural and functional implications. Biochem. Biophys. Res.Commun. 2017, 486, 992-997.

Acknowledgements. This work has been supported by Grant BFU2017-85723-P of the Spanish Ministerio de Ciencia, Innovación y Universidades, co-financed by FEDER. Jairo Salazar has been supported by a Predoctoral Fellowship of the Spanish Fundación Carolina (Madrid, Spain).

FC43-Ataxia related protein sacsin knockout disrupts the intermediate filaments network in glial cells

Murtinheira F.[1,2,3], Duarte-Olivenza C.[4], Costa-Teixeira M.B.[1,2,3], Letra-Vilela R.[1,2,3], Martin V.[4], Rodriguez C.[4], Valente C. [5], Oliva A.[3] and Herrera F.[1,2,3]

[1]Cell Structure and Dynamics Laboratory, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, [2] BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, [3] Instituto de Tecnologia Química e Biológica (ITQB-NOVA), Universidade Nova de Lisboa, Oeiras, Portugal, [4] Instituto Universitario de Oncología del Principado de Asturias (IUOPA) e Departamento de Morfología y Biología Celular, Facultad de Medicina, Universidade de Oviedo, Espanha, [5] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal.

Email address (Fernanda Murtinheira): fiferreira@fc.ul.pt

Background. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disorder, most commonly diagnosed in infants, characterized by progressive cerebellar ataxia, spasticity, motor sensory neuropathy and axonal-demyelination. ARSACS is caused by mutations in the *SACS* gene that lead to truncated or defective forms of the 520 kDa multidomain protein sacsin. Sacsin function has been exclusively studied on neuronal cells, where it regulates mitochondrial function and distribution and takes part in the normal polymerization of neurofilaments and vimentin. However, it remains unknown whether and how glial expression of mutant sacsin can contribute to ARSACS pathology. Therefore, our goal is to investigate how expression of mutant sacsin in glial cells affects its function and causes neuronal dysfunction and neurological phenotypes in ARSACS.

Methods. Sacsin expression in astrocytes was evaluated by western blot and immunocytochemistry. CRISPR/Cas9 system was used to delete sacsin in astroglia-like C6 rat glioma cells. Western blot, genome sequencing and immunocytochemistry analyses were performed to validate the knockout status. Mitochondrial morphology and distribution were analysed by fluorescent wide-field microscopy. Expression of intermediate filament proteins, namely glial fibrillar acidic protein (GFAP), nestin, vimentin and lamin B1 and the cytolinker protein plectin were assessed by western blot and immunocytochemistry.

Results. Our results show that sacsin is expressed in astrocytes and C6 rat glioma cells with a predominantly cytoplasmic and mitochondrial distribution. We generated and validated a stable sacsin knockout C6 cell line. Sacsin deletion in C6 cells induced an apparent mitochondrial depletion in the juxtanuclear area. The intermediate filaments GFAP, nestin and vimentin were accumulated in the same juxtanuclear area in about 40% to 70% of sacsin knockout cells, while only between 1%, and 5% of control cells displayed a collapsed network for these filaments. In sacsin knockout cells, an increased expression of the nuclear intermediate filament lamin B1 and the cytoskeletal cross-linking protein plectin was also observed.

Conclusions. Together, our findings provide insights that sacsin plays a role within glial cells as an organizer/enabler of intermediate filament networks and point at a possible role for astroglia in ARSACS dysfunctions.

FC44-Inflammation induces retinal pigment epithelium monolayer disruption and release of distinct populations of extracellular vesicles

Beatriz Martins[1,2], Teresa Rodrigues[1,2], José Ramalho[3], António Francisco Ambrósio[1,2,4], Henrique Girão[1,2], Rosa Fernandes[1,2,4]

[1]University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal; [2]University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal; [3]Centro de Estudos de Doenças Crónicas (CEDOC) and NOVA Medical School, Faculdade de Ciências Médicas (NMS/FCM); [4]AIBILI: Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal

Email address: beatriz.martins@uc.pt

Background: Age-related macular degeneration (AMD) is a retinal degenerative disease that affects the macula, constituting the leading cause of central vision loss in the elderly. Most of its phenotypical features are believed to be associated with the dysfunction of retinal pigment epithelium (RPE). In fact, the accumulation of damaged proteins in aged RPE is associated with disruption of proteolytic pathways and exocytic activity, with release of intracellular proteins via extracellular vesicles (EVs), which are important players in intercellular communication and can contribute to disease progression. However, the impact of their secretion by polarized RPE on outer blood retinal barrier (oBRB) breakdown remains largely elusive. Our aim was to clarify the role of inflammation on the loss of RPE integrity and to understand the relative role of EVs secreted by RPE in the loss of polarity and epithelial barrier disruption.

Methods: We used highly polarized porcine RPE primary cultures (pRPE) and porcine eyecups. To mimic the inflammatory conditions that characterize the disease, cells were treated with two distinct inflammatory stimuli, TNF (10 ng/mL) or LPS (100 ng/mL). Data were presented as mean ± S.E.M. Statistical significance was determined using Kruskal-Wallis test followed by Dunn's multiple comparisons test to compare among experimental conditions, in GraphPad Prism software.

Results: In pRPE, TNF and LPS did not affect the viability of the RPE cells. Once pRPE cells were cultured on transwell inserts, they developed a confluent monolayer and reached a relatively constant transepithelial resistance (TER) of about 300 Ω/cm^2. Treatment with both inflammatory stimuli significantly reduced the TER and induced disruption of tight junction (TJ) complexes, as demonstrated by decreased immunoreactivity at the plasma membrane of occludin. EVs isolated from the pRPE cells were distinctly characterized, presenting similar concentrations but with apical EVs being enriched in CD63 and basolateral EVs in CD81.

Regarding the porcine eyecups the inflammatory stimuli also induced a disruption of the TJ complexes, as detected by decreased ZO-1 and NaKATPase immunostaining, and an apparent disorganization of the epithelial monolayer, as revealed by scanning electron microscopy analysis. In the porcine eyecups under inflammatory conditions the release of EVs increased, which are enriched in CD63, CD81 and MMP-2. Treatment with TNF increases MMP-2 (active form) and MMP-9 (pro form) activity not only in the medium but also in the EVs isolated from porcine eyecups.

Conclusion: Overall our results show that inflammation induces loss of RPE monolayer integrity and release of different populations of EVs, which suggests that they may play an important role in the onset and progression of AMD.

Acknowledgments: Funding for this research was supported by the Global Ophthalmology Awards Program (GOAP), a Bayer sponsored initiative committed to supporting ophthalmic research across the world. Thanks are also due to Foundation for Science and Technology (FCT, Portugal) and Strategic Projects UID/NEU/04539/2019 (CNC.IBILI Consortium), and UIDB/04539/2020 and UIDP/04539/2020 (CIBB), COMPETE-FEDER (POCI-01-0145-FEDER-007440) and Centro 2020 Regional Operational Program: BRAINHEALTH 2020 (CENTRO-01-0145- FEDER-000008).

FC45-Involvement of MGP in African Spiny mice ear regeneration

M Vitorino[1,4], N Conceição[1,3,4], D Varela[1,2,3,4], G Tiscornia[1,5] and M L Cancela [1,3,4]

[1]Centre of Marine Sciences (CCMAR), University of Algarve/UALG, Faro, Portugal
[2]PhD in Biomedical Sciences, Faculty of Medicine and Biomedical Sciences, UALG, Faro, Portugal [3]Faculty of Medicine and Biomedical Sciences, and [4]Algarve Biomedical Centre, UALG, Faro, Portugal; [5]Clínica Eugin, Barcelona, Spain

Email address: lcancela@ualg.pt

The ability to regenerate damaged or missing organs has long been considered a primordial objective of modern medicine. Among vertebrates, it has been reported regeneration in urodele amphibians and, to a lower extent, in teleost fish such as zebrafish, which can regenerate several structures. In mammals, on the other hand, tissue regeneration is a rare event, responding to injury by wound healing through fibrotic scarring. In the last decade, an emerging model of mammalian epimorphic regeneration has arisen, the African spiny mouse (Acomys cahirinus) which is capable of non-fibrotic regeneration of extensive dermal wounds. Experiments previously performed in our laboratory showed that the spiny mice can regenerate full thickness ear holes up to 4 mm wide in around 2 months and that the regenerated tissue is not a fibrotic scar but a fully developed ear pinna with normal tissue structure, including cartilage, dermis, epidermis, adipose tissue, sebaceous glands, hair follicles and a well-developed capillary network. Matrix Gla protein (MGP) is a vitamin K-dependent protein, involved in preventing abnormal vascular and cartilage calcification by acting as a physiological inhibitor of calcification. In addition, MGP plays a role in regulating cell differentiation and angiogenesis. In this work we have identified the molecular structure of A. cahirinus MGP, which consists of 84 residues, like in humans. It includes a signal peptide, known to be required for its secretion into the ECM, a phosphorylation motif containing three serine residues located at the N-terminal end of the mature protein, separated from the remaining protein regions by a cleavage site (AXXF) followed by a γ-carboxylase recognition site (GGCX) and a Gla domain. The C-terminal RR cleavage site is also conserved. The posttranslational modification of five glutamic acid (Glu) residues into γ-carboxyglutamic acid (Gla) residues is central to MGP function and requires the GGCX activity with vitamin K as a cofactor. During A. cahirinus ear regeneration, MGP mRNA expression is down-regulated in the early stages of this process, but in the latest stages of cell differentiation, we observed a significant increase in MGP expression suggesting that it is involved in ear regeneration. These results confirm previous data on zebrafish and indicate that A. cahirinus may be an additional model of interest to study the function of MGP as a calcification inhibitor in mammals, in particular during wound formation and tissue regeneration.

FC46-MEK/ERK and cAMP/PKA mediate HIF-1α signalling during mitochondrial antioxidant induced osteoblastogenesis

Sunil Poudel[1,2,3], Gil Martins[1,2,3], M. Leonor Cancela[1,2,4], Paulo J. Gavaia,[1,2]

[1]Centre of Marine Sciences, University of Algarve, Faro, Portugal, [2]Faculty of Medicine and Biomedical Sciences (FMCB), [3]PhD Program in Biomedical Sciences, FMCB, University of Algarve, Faro, Portugal, [4]Algarve Biomedical Center, University of Algarve, Faro, Portugal

Email address: spoudel@ualg.pt

Background. Hypoxia-inducible factor 1α (HIF 1α) is one of the master regulators of hypoxia reactions and plays a crucial role in bone remodelling, homeostasis and metabolism. Oxidative stress alters the bone remodelling process by causing an imbalance between osteoclast and osteoblast activity. Mitochondria are the primary source of reactive oxygen species (mtROS) and the principal sites of ROS-induced damage. Mitochondrial dysfunction essentially influences osteoblasts through the regulation of mitophagy, apoptosis, and mitochondrial DNA damage.

Methods. MitoTempo (Mitochondria-targeted antioxidant) was used to investigate the effect of mtROS on osteoblast and osteoclast differentiation. Alkaline phosphatase activities and extracellular matrix mineralization capacity of MC3T3 cells during osteoblast differentiation and Tartrate-resistant acid phosphatase (TRAP) activity of pre-osteoclastic RAW264.7 cells during osteoclast differentiation were analyzed. To illustrate the molecular mechanism and cell signalling pathway, luciferase reporter assay was also performed during osteoblast differentiation.

Results. In vitro osteoblast differentiation show that MitoTempo increased alkaline phosphate activities and extracellular matrix mineralization during osteoblast differentiation. Similarly, MitoTempo was also highly effective in suppressing the RANKL/M-CSF-induced differentiation of RAW264.7 cells into TRAP-positive multinucleated osteoclasts. We found that the expression of HIF-1a pathway was increased during osteoblast differentiation under MitoTempo exposure. MitoTempo increased the luciferase activity of the MAP/ERK pathway while MAP/JNK was not affected. The pathway activity of cAMP/PKA was also increased upon MitoTempo exposure.

Conclusions. Our results suggest that mitochondrial ROS play a crucial role in bone remodelling. Accordingly, mitochondria-targeted antioxidant MitoTempo increases osteoblastogenesis and suppresses osteoclastogenesis. MitoTempo increases osteoblast differentiation via MAP/ERK-HIF-1a pathway, indicative of a HIF-1a dependent reprogramming of energy metabolism during osteoblast differentiation.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 766347- BioMedAqu, and by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020.

FC47-Pursuing the key residues behind Dis3L2 specificity

Costa SM[1], Arraiano CM[1], Matos RG[1], Viegas SC[1]

[1]Control of Gene Expression Lab, ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Email address: smma.costa@itqb.unl.pt

In humans, there are three members of the RNase II/RNB family of exoribonucleases, which can be distinguished according to the sequence conservation of their active site: Dis3, Dis3L (Dis3L1), and Dis3L2. Dis3L2 is involved in several cellular mechanisms, such as apoptosis, cellular differentiation, and proliferation, and mutations in the enzyme have been associated with Perlman syndrome and Wilms tumor formation in children (1). Distinct studies on Dis3L2 3'-5' exoribonuclease unraveled a novel eukaryotic RNA decay pathway that challenged the models already established. The first insight on the uridylation involvement in controlling the stability of poly(A)-containing mRNAs was reported in *Schizosaccharomyces pombe*, where it was shown that Dis3L2 activity is stimulated by the addition of untemplated uridine residues to the RNAs 3'-end (2). However, the precise mechanism of action of this enzyme is not yet fully understood.

Therefore, this work aims to characterize the amino acid residues that distinguish Dis3L2 from its family homologs regarding its substrate specificities, namely the preference for uridine residues. This approach will help us to understand its mechanism of action and its function in different eukaryotic cells.

In this work, fission yeast Dis3L2 was used to construct mutant proteins with single amino acid substitutions whose expression was induced in *E. coli*. The purified proteins were used in several assays to assess their exonucleolytic activity over different radioactively labeled RNA substrates. We have purified active Dis3L2 mutants, and we have tested their preference for poly(U), by using uridylated and non-uridylated endogenous substrates, and furthermore their ability to cut double-stranded RNAs. So far, we have found that six mutant ribonucleases continue to exhibit a preference for oligo(U)-tailed RNAs, despite showing discrete differences regarding the degradation of different RNAs.

References

1. Saramago M, da Costa PJ, Viegas SC, Arraiano CM. 2019. The Implication of mRNA Degradation Disorders on Human DISease: Focus on DIS3 and DIS3-Like Enzymes. Adv Exp Med Biol 1157:85–98.
2. Malecki M, Viegas SC, Carneiro T, Golik P, Dressaire C, Ferreira MG, Arraiano CM. 2013. The exoribonuclease Dis3L2 defines a novel eukaryotic RNA degradation pathway. EMBO J 32:1842–1854.

FC48-The role of understudied post-translational modifications on STAT3 behavior and function

Martins-Alves D.[1,2], Diallo M.[1,2,3], Letra-Vilela R.[1,2,3], Murtinheira F.[1,2,3], Herrera F.[1,2,3]

[1]Laboratório de Estrutura e Dinâmica Celular, Faculdade de Ciências, Universidade de Lisboa, Portugal. [2]BioISI - Instituto de Biossistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, Portugal. [3]Unidade de Investigação MOSTMICRO, Instituto de Tecnologia Química e Biológica (ITQB-NOVA), Universidade Nova de Lisboa, Portugal.

Email address: dani.alves98@gmail.com

Background. Signal Transducer and Activator of Transcription 3 (STAT3) is a pleiotropic transcription factor that plays key roles in development, immunity, response to stress/damage and cancer. Its activity is largely regulated by specific post-translational modifications (PTMs) and protein-protein interactions. Most studies focus only on the phosphorylation at Y705 and/or S727, neglecting almost 80 identified PTMs. Furthermore, it is unlikely that all STAT3 molecules are modified exactly in the same residues, existing different pools of STAT3 molecules. We have recently described that asymmetrically modified STAT3 dimers showed changes in their behavior and function. We aimed at determining the influence of understudied PTMs and asymmetric modifications on STAT3 homo- and heterodimerization, intracellular distribution and function.

Methods. We recently developed a Venus-STAT3 bimolecular fluorescence complementation assay to visualize and study STAT3 homo- and heterodimers in living cells. In BiFC assays two proteins of interest are fused to two complementary non-fluorescent halves of a fluorescent reporter, in this case Venus. If the proteins of interest interact, they bring the reporter halves back together, reconstituting the functional fluorophore. Fluorescence is therefore proportional to the dimerization of the proteins and can be easily measured by flow cytometry or microscopy. Venus-STAT3 BiFC constructs were modified by site-directed mutagenesis, mutating phosphorylatable residues (Y, S, T) to structurally similar, non-phosphorylatable residues (F, A). These constructs were transfected into STAT3 knockout HeLa cells to avoid the possible interference of endogenous STAT3.

Results. The PTM mutant Venus-STAT3 constructs were tested in the corresponding KO HeLa cells for their expression and fluorescence levels, as well as the intracellular distribution and transcriptional activity of STAT3 homodimers, STAT3/GRIM19 and STAT3/HIF1α heterodimers. We observed alterations in the behavior of STAT3 PTM mutant constructs versus non-mutant STAT3, in both symmetric and asymmetric STAT3 dimers. The transcriptional activity of these dimers also changed, and so did the pattern of interaction with GRIM19 and HIF1α.

Conclusions. Our results indicate that specific understudied PTMs can regulate STAT3 intracellular localization, activity and interaction with other proteins. Since STAT3 has very diverse biological roles, understanding how its activity its regulated could be very relevant for the development of therapies for human diseases such as immune disorders, neurodegeneration and cancer.

FC49-Hydrogen Peroxide as an Intercellular Redox Signaling Molecule in the Brain: Diffusivity, Uptake and Removal

Ledo, A.[1, 2], Fernandes, E. [1], Laranjinha, J. [1, 2] Barbosa, R.M.[1, 2]

[1]University of Coimbra, Faculty of Pharmacy, Portugal, [2]Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Email address: analedo@ff.uc.pt

Background. Hydrogen peroxide – H_2O_2 – is recognizably a major biological oxidant which plays a pleiotropic role in redox regulation of biological activities. Its intracellular concentration is maintained in the low nM range through efficient removal systems. Despite being a strong oxidant, its high activation energy limits its reactions with most biological molecules, with cysteine residues in specific proteins being particularly favored. In the brain, a role for H_2O_2 as an intercellular redox signaling molecule and neuromodulator has become evident in the regulation of neuronal polarity, connectivity, synaptic transmission and tuning of neuronal networks.

Methods. In the present study we used novel ruthenium-purple modified carbon fiber electrodes (CFM-RP-Nafion®) to monitor the concentration of H_2O_2 in striatum slices and *in vivo*. These sensors display electrocatalytic activity for the reduction of H_2O_2 at −0.1 V vs. Ag/AgCl, with an average sensitivity of 0.98 ± 0.37 μA cm^{-2} μM^{-1} and limit of detection of 70 ± 40 nM, allowing selective measurement of H_2O_2 with high temporal and spatial resolution in brain. Using these sensors implanted into the brain tissue, we investigated the diffusibility of extracellular H_2O_2 and determined the $t_{1/2}$ as well as the *in vivo* diffusion coefficient of H_2O_2. We also determined the contribution of enzyme systems (catalase – CAT; glutathione peroxidase – GPx; and peroxiredoxin - Prx) in modulating extracellular H_2O_2 concentration dynamics and decay.

Results. We found that both in brain slices and in vivo, exogenously applied (*puff*) H_2O_2 is rapidly removed from the extracellular space though energy-dependent mechanisms. In brain slices, the signal decay followed a 1st order exponential decay function from which the decay constant was found to be k = 0.32 ± 0.02 s^{-1}, with a decay half-time of $t_{1/2}$ = 2.51 ± 0.14 s. Furthermore, the rate of decay was decreased by inhibition of GPx and Prx, but not CAT. Data obtained *in vivo* in the rat striatum revealed similar decay kinetics, with k = 0.34 ± 0.01 s^{-1}, corresponding to an average $t_{1/2}$ = 2.19 ± 0.07 s. However, in vivo all enzymatic systems contribute to H_2O_2 breakdown, suggesting a role for erythrocyte-contained catalase in shaping H_2O_2 concentration dynamics in the brain. Mathematical fitting of the experimental data obtained to the equation that describes diffusion from a spherical source allowed us to estimate the effective diffusion coefficient (D*) of H_2O_2 in vivo to be 2.49 x 10^{-5} ± 0.13 x 10^{-5} cm^2 s^{-1} with an average inactivation constant of λ= 0.19 +/- 0.01 s^{-1}. From the Einstein-Smoluchowski equation we found that, within the half-time of H_2O_2 in the extracellular space, this biological oxidant may travel a distance of over 100 μm.

Conclusions. Our data support the notion that H_2O_2 can act as a volume signaling molecule in vivo in the brain. Furthermore, H_2O_2 present in the extracellular space in removed by intracellular enzymatic systems, in neural cells by GPx and Prx, and in circulating erythrocytes by CAT as well. The most likely rout of entry into cells are aquaporins coined peroxiporins.

Acknowledgements: POCI-01-0145-FEDER-028261, UIDB/04539/2020

FC50-Autosomal recessive spastic ataxia of Charlevoix-Saguenay: A 3D epidermal model to understand sacsin function in the skin

Costa-Teixeira. M.B.[1,2]; Murtinheira.F[2,3], Lopes-Ventura, S[1].; Herrera, F[2,3].; Oliva, A[1]

[1]Biomolecular Diagnostic Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal; [2]Cell Structure and Dynamics Laboratory, BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; [3]MOSTMICRO Research Unit, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal.

Email address (M.B. Costa-Teixeira): beatriz.teixeira@itqb.unl.pt

Background. The Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)[1] is a rare, early-onset neurological disease that was first described in Quebec, Canada, but cases have been reported worldwide. Patients suffer from spasticity and lack of muscle coordination, resulting in an early wheelchair dependence and premature death. ARSACS is caused by mutations in the *SACS* gene that lead to sacsin loss-of-function. Although sacsin is highly expressed in the central nervous system, ARSACS patients also show skin alterations, and our data indicate that primary keratinocytes express high levels of the protein.

Methods. Using reconstructed 3D human skin protocols, we aim to develop an epidermal model that replicates ARSACS features, to further understand sacsin function and to help the design of new diagnostic and therapeutic strategies. For this, we first tried to produce 3D epidermis from human keratinocyte cell lines HaCaT [2] and N/TERT-1 [3].

Results. We successfully developed 3D epidermal models using both keratinocyte cell lines. However, the model using the HaCaT cell line presented a few limitations. This cell line was unable to fully recreate the multi-layer architecture of native skin, without the formation of the outermost layer, the *stratum corneum*. Moreover, the HaCaT cell line showed a very low expression of sacsin, which did not change upon exposure to lentiviral shRNAs against sacsin. On the other hand, the N/TERT-1 cell line generated a stratified epidermis with all the layers present and expressed sacsin in similar amounts to primary human keratinocytes.

Conclusions. These findings suggest that the N/TERT-1 cell line has more potential to produce an epidermal skin model with an ARSACS phenotype in the following experiments. This model can become an important tool to further understand sacsin function outside the central nervous system. We hope to advance our knowledge of ARSACS disease by contributing to the identification of new molecular markers and possible therapeutic targets to help diagnose and treat this debilitating and lethal disease.

References.

[1] J. Bouchard, A. Barbeau, R. Bouchard, and R. Bouchard, “Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay,” Can. J. Neurol. Sci., vol. 5, no. 1, pp. 61–69, 1978.

[2] P. Boukamp, R. T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, and N. Fusenig E., “Normal Keratinization in a Spontaneously Immortalized Aneuploid Human Keratinocyte Cell Line,” J. Cell Biol., vol. 106, no. March, pp. 761–771, 1988.

[3] M. A. Dickson et al., “Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics.,” Mol. Cell. Biol., vol. 20, no. 4, pp. 1436–47, 2000.

FC51-Proteomic analysis as a tool to identify key differentially expressed proteins during human sperm cryopreservation

Corda, PO[1]; Silva, JV[1,2,3]; Alves, MG[1]; Fardilha, M[2]

[1]Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal; [2]Institute of Biomedicine (iBiMED), Department of Medical Sciences, University of Aveiro, Aveiro, Portugal; [3]i3S - Instituto de Investigação e Inovação em Saúde - University of Porto, Porto, Portugal

*Email address: pedrocorda@ua.pt

Introduction: Sperm cryopreservation is a key procedure in reproductive medicine worldwide being useful to preserve male fertility in pathological scenarios and in some male infertility conditions. Currently, two conventional freezing techniques are used in sperm cryopreservation: (a) slow freezing (SF) and (b) rapid freezing (RF). Notwithstanding, the cryopreservation protocols are still not ideal inducing significant changes at spermatozoon structural and molecular levels. Proteomics emergence brought a new perspective on sperm physiology, allowing the evaluation of the protein profile in certain physiological/pathological contexts. Through proteomic data, we identified key differentially expressed proteins (DEPs) in freeze-thawed human spermatozoa that can be potential cryopreservation quality biomarkers or modulation targets.

Methods: We selected proteomic studies that compared fresh controls with human sperm cryopreserved from normozoospermic donors and collected the DEPs in two groups: SF and RF groups. For each group, an enrichment analysis (biological process, cellular compartment and KEGG pathways) was performed and protein-protein interactions (PPIs) were collected in the String database. Then, integrative networks were built, using the 10 most significant terms in each category and PPIs, to identify the proteins with the highest degree of connectivity.

Results: We collected 160 DEPs for SF group and 555 DEPs for RF group. DEPs from SF group were mainly related to translation, protein-targeting, viral infection, antigen processing and metabolic processes. On the other hand, DEPs from RF group were associated with exocytosis, cell cycle and differentiation, metabolic processes, signalling pathways, protein processing and trafficking and translation. We collected 54 and 607 PPIs for SF and RF groups, respectively. In each integrative network, we identified 10 key proteins. From those, four proteins are common to both groups.

Conclusion: The use of proteomic data in combination with a bioinformatic workflow allowed the rapid identification of four potential key DEPs in freeze-thawed human spermatozoa. Future studies should be performed to validate experimentally the role of those proteins as cryopreservation quality biomarkers or modulation targets.

FC52-Involvement of *Znf687* during osteoblast proliferation and differentiation

Varela D[1,2], Conceição N[1,3,4], Cancela ML[1,3,4]

[1]Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal; [2]PhD in Biomedical Sciences, Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal; [3]Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal; [4]Algarve Biomedical Centre, University of Algarve, Faro, Portugal

Email address: a44771@ualg.pt

Background. *ZNF687* gene encodes a protein belonging to the C2H2 zinc finger protein family that may be involved in bone metabolism. Mutations in *ZNF687* have been associated with severe cases of Paget's Disease of Bone (PDB), the second most common metabolic bone condition, characterized by an increased bone resorption mediated by the numerous, giant and hyperactive osteoclasts, followed by an abnormal and excessive osteoblast-mediated.bone formation. As a result, affected bones of PDB patients are disorganized, enlarged and deformed, therefore prone to fracture. Although ZNF687 function is still poorly understood, studies showed that it is highly expressed during the regeneration of caudal fins in zebrafish and overexpressed in peripheral blood mononuclear cells samples derived from PDB-affected individuals. Thus, the aim of our work was to investigate the role of ZNF687 throughout osteoblast proliferation and differentiation using a mouse-derived osteoblast precursor cell line (MC3T3-E1).

Methods. To achieve our goal, MC3T3-E1 were differentiated into mature osteoblasts by treating the cells every 2 days for 28 days with an osteogenic cocktail consisting of ascorbic acid (50 µg/mL) and β-glycerophosphate (10 mM). At specific differentiation times, mineralization (calcium deposition) was detected and quantified by alizarin red staining, total RNA was extracted and levels of *Znf687* gene expression and markers of osteogenic differentiation were determined through real-time PCR.

Results. The MC3T3-E1 cells treated with osteogenic differentiation medium showed a more intense red staining of mineralized bone matrix than the untreated control cells. That was even more evident at day 28 of treatment where there was a significant calcium deposition shown by the higher number of alizarin red-stained mineralized nodules. Accordingly, the quantification of the mineralized matrix (calcium-bound alizarin red) showed that, in fact, there was more mineral deposition by cells treated with osteogenic differentiation medium. Our results showed a higher expression of bone markers during the osteogenic treatment compared to the control. *Znf687* expression was downregulated throughout osteoblast differentiation, i.e., its expression was significantly lower in differentiated osteoblasts than in their undifferentiated precursors. No differences were observed in *Znf687* expression during osteoblast proliferation.

Conclusions. Our work suggests that Znf687 plays a role in osteoblast differentiation but not in osteoblast proliferation. More studies are needed to understand the mechanisms involved in *Znf687* expression during this process.

References.

Divisato G, et al. ZNF687 Mutations in Severe Paget Disease of Bone Associated with Giant Cell Tumor. Am J Hum Genet. 2016, 98:275-86.

Feng X and McDonald JM. Disorders of Bone Remodeling. Annu Rev Pathol. 2011, 6:121-145.

Funding. This work received national funds from the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/04326/2020 (CCMAR). Varela D is recipient of PhD fellowship from FCT (ref: SFRH/BD/141918/2018).

S6/S8/S9 - SBBQ-FUNCTIONAL GENOMICS AND SYSTEMS BIOLOGY / SEBBM-PROTEINS IN HEALTH AND ENVIRONMENT / CHEMICAL BIOLOGY, DRUG DISCOVERY AND DEVELOPMENT

FC53	Patrícia Apura	A short trip to the world of Pseudomonas putida's ribonucleases
FC54	Sandra Viegas	Novel non-coding RNAs whose expression is relevant for the growth of Pseudomonas putida in a bioreactor
FC55	Ana Silva	Cytochrome c maturation System III: can an eukaryotic system produce multiheme cytochromes?
FC56	Joana Madjarov	BioCat: Investigating the electron uptake mechanism of Sporomusa ovata for microbial electrosynthesis of acetate from CO2
FC57	Gil Fraqueza	Ca2+-ATPase inhibition studies by polyoxometalates
FC58	Americo Alves	Chiral α-Alkylidene-Substituted β-Lactams and γ-Lactams: Synthesis and Anticancer Activity
FC59	Mariana Marques	Development of a canine skin analog
FC60	Carlos Gastalho	Development of different multifunctional chitosan nanocarriers with pharmacological activity for human skin wounds healing
FC61	Dorinda Marques da Silva	A graphical journey through the MBStox project
FC62	Nazua L.Costa	Electrografted anthraquinone as a probe to measure pH changes at biofilm-anode interface in a wastewater operating microbial fuel cell
FC63	Inês B. Trindade	Ghost hunting: FhuF - a ferric-siderophore reductase of unknown structure
FC64	Vânia Moreira	Development of a LSPR-based biosensor for the SARS-CoV-2 detection
FC65	Custódia Fonseca	INHIBITION OF THE Ca2+-ATPase BY GOLD (I, III) COMPOUNDS
FC66	Constança Pais do Amaral	Bioactive peptides isolated from European amphibian skin secretions with possible anticancer effect

Chairs: Ricardo Louro (ITQB NOVA), João Ramalho (UC)

FC53-A short trip to the world of *Pseudomonas putida*'s ribonucleases

Apura, P.[1], de Lorenzo, V.[2], Gonçalves, L. G.[1], Martínez-García, E.[2], Arraiano, C. M.[1], and Viegas, S. C.[1]

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, EAN, Oeiras, 2780-157, Portugal; [2]Systems Biology Program, Centro Nacional de Biotecnologia, CSIC, Madrid, Spain.

Email address: papura@itqb.unl.pt

Ribonucleases (RNases) are important effectors on post-transcriptional regulation and coordinators of bacterial adaptation to stress. The aim of this work is to shed light on the importance of ribonucleases function for the versatile metabolism of *Pseudomonas putida*. This gram-negative saprophytic bacterium is generally recognized as a laboratory work model of environmental bacteria and is endowed with a diversity of metabolic and stress endurance traits that make it an ideal chassis for biotechnological needs. Bacterial RNases have been most extensively studied in the model organism *Escherichia coli*, however, in *P. putida* that information is still scarce. In this work we attempted to inspect the cellular effects of the absence of specific ribonucleases in *P. putida* covering the bulk of possible RNA cleavages in this microorganism and looking at them in connection to its conspicuous environmental endurance. Following this line, we have constructed single mutants for five different *P. putida* ribonucleases, two exoribonucleases (PNPase and RNase R) and three endoribonucleases (RNase E, RNase III and RNase G) and analysed the physiological, phenotypical and metabolic costs of their absence. The impact of these mutants is tested in terms of growth, motility and morphology. In addition, the effects of different chemicals triggering oxidative stress were tested, as well as proxies of toxicants present in the natural environments typically inhabited by this microorganism. Our data highlights RNases and, consequently, RNA transactions as a major control layer that enables *P. putida* thrive in a variety of stressful conditions. Moreover, the physiological response of *P. putida* in the absence of each enzyme differs, in some cases, from the one previously observed in *E. coli*, revealing evident differences in the metabolism of these two bacteria but also different enzymatic functions of the ribonucleases in each bacterial landscape.

References.

Apura, P., et al., Ribonucleases control distinct traits of *Pseudomonas putida* lifestyle, Environmental Microbiology, (2021) 23(1), 174–189.

Apura, P. *et al.*, The world of ribonucleases from pseudomonads: a short trip through the main features and singularities. Microbial Technology. Special Issue article (2021).

FC54-Novel non-coding RNAs whose expression is relevant for the growth of *Pseudomonas putida* in a bioreactor

Viegas SC[1], Pobre V[1], Graça-Lopes G[1], Apura P[1], Domingues S[1], Saramago M[1], Ankenbauer A[2], Takors R[2] and Arraiano CM[1]

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal; [2]Institute of Biochemical Engineering, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany

Email address: sviegas@itqb.unl.pt

Background. *Pseudomonas putida* is a ubiquitous Gram-negative bacterium with an extraordinary metabolic versatility and robustness, which makes it a platform of choice for the engineering of biotransformation reactions with applications in biotechnology, agriculture, and bioremediation. This will ultimately contribute to a greener and more sustainable economy.

In industrial fermentation processes it is critical to maintain a robust and prolonged productivity under stressful conditions. With the use of bacterial functions of increasing complexity came the awareness that different bacterial microorganisms respond differently in accordance with their regulatory layers. Small non-coding RNAs (ncRNAs) have arisen as an effective strategy to improve strain's tolerance. In nature, ncRNAs exist as a heterogeneous group of molecules that are involved in controlling a variety of stress responses. These functional regulatory molecules are usually not translated and can interact with RNA, DNA, proteins and small molecules regulating many different cellular functions.

Manipulation of bacterial small non-coding RNAs (ncRNAs) has been recognized as an effective tool to improve the production of industrial compounds. The natural versatility of RNA regulators has also inspired the design and construction of several ncRNA based synthetic devices to modulate heterologous protein expression.

Although a few ncRNAs have been characterized in the genus Pseudomonas, there is a general dearth of knowledge on the specific functional roles of ncRNAs in biotechnologically relevant microorganisms, such as P. putida. Furthermore, we realized that there is scarce information regarding which ncRNAs are induced when bacteria face the stress of growing in industrial bioreactors. This work aims to address this gap, with the identification of novel and potentially important ncRNAs for the adaptation of this model platform to large scale production conditions.

Methods. We have performed a general transcriptomic analysis approach to investigate the differential expression of *P. putida* transcripts induced under stress conditions experienced in an industrial bioreactor. For that, *P. putida* was cultivated in a two-compartment scale-down bioreactor that simulates large-scale industrial bioreactors. An RNA-seq of samples collected at distinct locations and time-points was performed and the newly identified ncRNAs are being validated by Northern blot analysis. We have done a bioinformatic analysis of the fold change in expression levels of the identified ncRNAs in different locations and time-points of the bioreactor setting, which is now being quantitatively evaluated by RT-qPCR.

Results. 725 novel ncRNAs were predicted in our analysis (Pobre *et al.*, 2020). We also found that the expression of these ncRNAs was not constant throughout the bioreactor, showing different patterns of expression with time and position.

Conclusions. Our results will unveil the ncRNAs important for *P. putida* during stress adaptation. Characterization of these regulators can bring a valuable information to be applied in biotech applications to improve stress tolerance.

References.

Pobre, V., et al. (2020) Prediction of novel non-coding RNAs relevant for the growth of *Pseudomonas putida* in a bioreactor. Microbiology 166, 149-156.

FC55-Cytochrome *c* maturation System III: can an eukaryotic system produce multiheme cytochromes?

Silva, AV[1]; Firmino, MO[1]; Costa, NL[1]; Louro, RO[1]; Paquete, CM[1]

[1]Instituto de tecnologia química e biológica António Xavier (ITQB-Nova)

Email address: anavsilva@itqb.unl.pt

Background. *C*-type cytochromes are metalloproteins that contain one or more hemes covalently bound to the polypeptide chain. These proteins are ubiquitous and fundamental for biological processes in the cell across all domains of life, including respiratory processes, catalysis, signaling and apoptosis. Given their importance, the process that leads to their formation in nature is equally important. In fact, the deficiency in cytochrome-*c* maturation has been identified as the sole cause of the genetic disease MLS (microphthalmia with linear skin defects). The covalent attachment of the heme to the apo-protein requires a maturation machinery. In the case of eukaryotic organisms, the system responsible for the process is composed of only one enzyme - CcHL or HCCS. So far, this system has only been described to produce mono-heme cytochromes. This work is focused on understanding this maturation system and, more specifically, the requirements for substrate recognition by this enzyme.

Methods. To address the recognition mechanisms, the gene for the bacterial cytochrome STC (small tetraheme cytochrome *c* from *Shewanella oneidensis* MR-1) was cloned in a plasmid harboring CcHL. Furthermore, the previously described CcHL recognition sequence was inserted by site directed mutagenesis in two different heme binding positions, generating the mutants STC-H1, STC-H2 and STC-H12. These plasmids were then transformed in *Escherichia coli* BL21 and the different mutants were expressed, purified and analyzed using UV-visible spectroscopy, Mass Spectrometry and NMR. As a control, all the proteins were also expressed with the native System I.

Results. CcHL can recognize the apo-cytochrome of a bacterial multi-heme protein when the recognition sequence is present either in the first or in the second heme binding position. However, the enzyme does not produce a correctly folded protein and it does not seem to be able to insert more than one heme, even when two recognition sequences are present.

Conclusions. It was shown for the first time that CcHL can recognize multiheme *c*-type cytochromes, however, the correct folding of the cytochrome is most likely dependent on the presence of all hemes and therefore, could not be achieved. It was also possible to observe that the presence of the recognition sequence is not the only requirement for heme insertion.

FC56-BioCat: Investigating the electron uptake mechanism of *Sporomusa ovata* for microbial electrosynthesis of acetate from CO_2

Madjarov, J., Paquete C.M., Louro, R. O.

Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa,
Av. da República (EAN), 2780-157 Oeiras, Portugal

Email address: joana.madjarov@itqb.unl.pt

Background. Microbial electrosynthesis (MES) is an intriguing new technology to produce high value chemicals from CO_2 and electricity. It relies on acetogenic bacteria like *Sporomusa ovata*, which can accept electrons from an electrode and use carbon dioxide to synthesize acetate (figure 1)[1].

Within this dynamic field of MES research, one of the main unsolved questions deals with the mechanism of electron uptake of bacteria from an electrode. The EU funded project BioCat, which shall be introduced here, contributes to answer this by detecting and analyzing the involved proteins and their interactions on a molecular level. So far, the fundamentals of electron uptake of the well-studied model organism *Shewanella oneidensis* have been shown. There, a porin-cytochrome c complex [2] is responsible for the electron transport which can transfer electrons in both directions [3]. But *Shewanella* is not autotrophic and thus is not able to metabolize carbon dioxide.

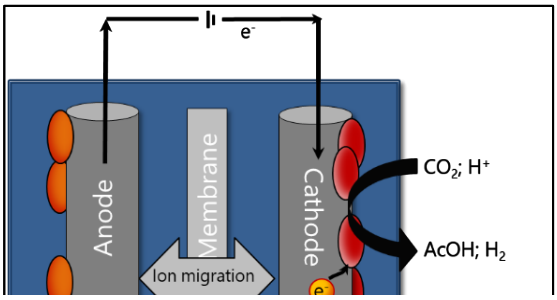


Figure 1: Microbial electrosynthesis cell for the production of Acetate

Methods. To detect and analyze the electron uptake proteins of the acetogen *Sporomusa ovata*, a strictly anaerobic reactor was designed which performs microbial electrosynthesis of acetate. It will be operated with graphite electrodes and medium 311[4]. To supply the needed carbon dioxide it is gently purged with N_2:CO_2 in the ratio 80:20. The experiments are performed potentiostatically with a three electrode setup to exclude limitations caused by the anode performance. The expressed proteins and the acetate production on the cathode will be analyzed as functions of operating parameters. Subsequently *Sporomusa o.* will be treated with mutagens to induce mutations aiming the optimization of electron uptake rates.

Results. The project BioCat is kicking off right now and so far, the reactor has been developed based on the widespread H-Cell reactor. At the time, the SPB congress will take place, there will be first results on electron uptake rates and acetate production of *Sporomusa o.*

References.

[1] P. Tremblay and T. Zhang, “Electrifying microbes for the production of chemicals,” vol. 6, no. March, pp. 1–10, 2015.
[2] M. A. TerAvest and C. M. Ajo-Franklin, “Transforming exoelectrogens for biotechnology using synthetic biology,” *Biotechnol. Bioeng.*, vol. 113, no. 4, pp. 687–697, Apr. 2016.
[3] N. M. Tefft and M. A. Teravest, “Reversing an Extracellular Electron Transfer Pathway for Electrode-Driven Acetoin Reduction,” *ACS Synth. Biol.*, vol. 8, no. 7, pp. 1590–1600, 2019.
[4] “311. Sporomusa Medium (Betaine),” *Dsmz*, 2015.

FC57-Ca^{2+}-ATPase inhibition studies by polyoxometalates

Fraqueza, G.[1], Gumerova, N. I.[2], Rompel, A.[2], Aureliano, M.[3]

[1]CCMar, ISE, Universidade do Algarve, Portugal, [2]Universität Wien, Fakultät für Chemie, Institut für Biophysikalische Chemie, Austria, [3]CCMar, FCT, Universidade do Algarve, Portugal

Email address: (gfraque@ualg.pt)

Background. Polyoxometalates (POMs) are a kind of inorganic cluster metal complexes with various biological activities, such as antibacterial, anticancer, anti-diabetes, antivirus and anti-Alzheimer's disease among others. Their action mechanism at the molecular level is basically unknown. POMs have shown inhibitory effect on several enzyme families, such as α-glucosidase, tyrosinase, protein kinase or exonuclease. POMs also inhibited sialyltransferase, thiotransferase, deacetylase and virus reverse enzyme in different degrees. To study the inhibitory effects of POMs on various enzymes will contribute to the application of polyoxometalates in biomedicine.

Our research group has studied and analysed the inhibitory effects of nine different polyoxotungstates (POTs) on Ca^{2+}-ATPase from skeletal muscle.

Methods. For Ca^{2+}-ATPase inhibition, an in vitro study was performed and the strongest inhibitors were determined to be the large heteropolytungstate $K_9(C_2H_8N)_5[H_{10}Se_2W_{29}O_{103}]$ (Se_2W_{29}) and the Wells-Dawson type POT $K_6[\alpha\text{-}P_2W_{18}O_{62}]$ (P_2W_{18}) exhibiting IC_{50} values of 0.3 and 0.6 μM, respectively [1]. Promising results were also shown for the Keggin-based POTs $K_6H_2[CoW_{11}TiO_{40}]$ ($CoW_{11}Ti$, IC_{50} = 4 μM) and $Na_{10}[\alpha\text{-}SiW_9O_{34}]$ (SiW_9, IC_{50} = 16 μM), $K_{14}[As_2W_{19}O_{67}(H_2O)]$ (As_2W_{19}, IC_{50} = 28 μM) and the lacunary Wells-Dawson $K_{12}[\alpha\text{-}H_2P_2W_{12}O_{48}]$ (P_2W_{12}, IC_{50} = 11 μM), whereas low inhibitory potencies were observed for the isopolytungstate $Na_{12}[H_4W_{22}O_{74}]$ (W_{22}, IC_{50} = 68 μM) and the Anderson-type $Na_6[TeW_6O_{24}]$ (TeW_6, IC_{50} = 200 μM). Recently three new POTs were studied, including two lacunary Wells-Dawson POTs $Na_{12}[\alpha\text{-}P_2W_{15}O_{56}]\cdot 24H_2O$ (P_2W_{15}), $K_{10}[P_2W_{17}O_{61}]$ $20H_2O$ (P_2W_{17}), and the Preyssler type $(NH_4)_{14}[NaP_5W_{30}O_{110}]\cdot 31H_2O$ (P_5W_{30}), all strong inhibitors of Ca^{2+}-ATPase with IC_{50} values < 1 μM.

Results. Regarding the mode of interaction between POTs and Ca^{2+}-ATPase, the type of inhibition was determined for some of the POTs and for all of them it was observed that POTs exhibited a mixed type inhibition. Furthermore, speciation of POT clusters at physiological pH 7.0 and in water was investigated by ^{31}P-NMR analysis in order to address cluster species responsible for the interaction. A solution studying the most promising ATPase inhibitor, P_5W_{30}, revealed that immediately after dissolving the Preyssler POT three anions are present in solution: intact Preyssler (P_5W_{30}), intact Wells-Dawson (P_2W_{18}) and mono-lacunary Wells-Dawson anions (P_2W_{17}).

Conclusions. These results reveal the high potential of some POTs to act as P-type ATPase inhibitors. As P-type ATPases represent pharmacologically important targets due to their important role in health and disease, the here reported bioactive POTs should be considered as possible future metallodrugs.

References

[1] Gumerova N. I., Krivosudsky L., Fraqueza G., Breibeck J., Al-Sayed E., Tanuhadi E., Bijelic A., Fuentes J., Aureliano M., Rompel A. (2018) The P-type ATPase inhibiting potential of polyoxotungstates. Metallomics 10 (2): 287-295. doi:10.1039/c7mt00279c

FC58-Chiral α-Alkylidene-Substituted β-Lactams and γ-Lactams: Synthesis and Anticancer Activity

Américo J. S. Alves[1], Nuno G. Alves[1], Mafalda Laranjo[2,3,4], M. Filomena Botelho[2,3,4] and Teresa M. V. D. Pinho e Melo[1]

1University of Coimbra, Coimbra Chemistry Centre and Department of Chemistry, 3004-535 Coimbra, Portugal; 2Institute of Biophysics and Institute for Clinical and Biomedical Research (iCBR), Area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal; 3Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, 3000-548 Coimbra, Portugal; 4Clinical and Academic Centre of Coimbra, 3000-548 Coimbra, Portugal

Email address: americo.jsa.92@gmail.com

Cancer is one of the leading causes of death being responsible for nearly 10 million deaths annually and remains as one of the most difficult diseases to treat. The rapid development of drug-resistant cancers, and the low specificity of some anticancer agents with the associated side effects are some of the major obstacles to overcome. Thus, one of the main challenges of medicinal chemists around the world is the development of novel anticancer agents. Despite the major area of research of β-lactams focuses on antibacterial activity, a wide range of β-lactamic compounds were discovered with diverse biological activities.[1] Furthermore, the alkylidene-lactam moiety has been associated with anticancer activity.[2] In this communication, chiral alkylidene-substituted β-lactams derived from 6-aminopenicillanic acid and the corresponding γ-lactam derivatives were synthesized and screened for their *in vitro* activity against four human cancer cell lines (melanoma, esophageal, lung and fibrosarcoma carcinoma). The novel chiral alkylidene-γ-lactams were synthesized through a multistep strategy starting from D-penicillamine. The *in vitro* assays allowed the identification of 4 compounds with IC_{50} values under 10 μM. Further studies were carried out with the more promising compounds in order to determine the induced mechanism of cell death, reactive oxygen species generation and inhibition of matrix metalloproteinases, unveiling the potential of alkylidene-β-lactams as anticancer agents (Figure 1).

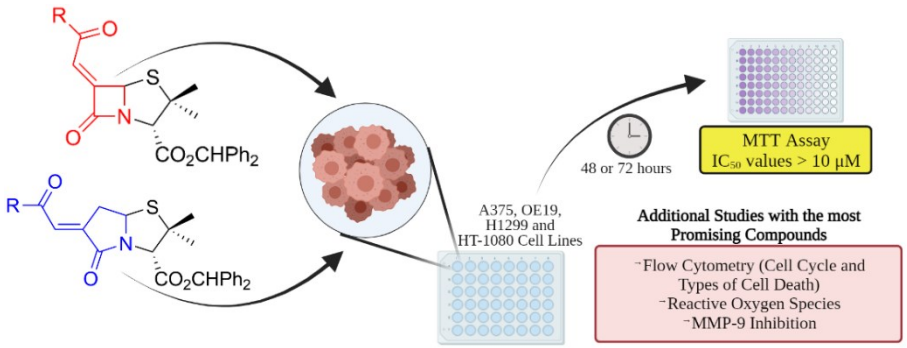


Figure 1.

References

[1] a) N. Arya, A. Y. Jagdale, T. A. Patil, S. S. Yeramwar, S. S. Holikatti, J. Dwivedi, C. J. Shishoo and K. S. Jain, *Eur. J. Med. Chem.* **2014**, 74, 619-656; b) P. D. Mehta, N. P. S. Sengar and A. K. Pathak, *Eur. J. Med. Chem.* **2010**, 45, 5541-5560; [2] a) G. Veinberg, I. Shestakova, M. Vorona, I. Kanepe and E. Lukevics, *Bioorg. Med. Chem. Lett* **2004**, 14, 147-150; b) A. Albrecht, Ł. Albrecht and T. Janecki, *Eur. J. Med. Chem.* **2011**, 2011, 2747-2766.

Acknowledgements. The authors thank Coimbra Chemistry Centre (CQC), supported by the Portuguese Agency for Scientific Research, "Fundação para a Ciência e a Tecnologia" (FCT) through project UIDB/00313/2020 and UIDP/00313/2020, co-funded by COMPETE2020-UE. The authors also thank CIBB, funded by National Funds via FCT through the Strategic Project UIDB/04539/2020 and UIDP/04539/2020. Américo J. S. Alves thank FCT for fellowship SFRH/BD/128910/2017. The authors also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt).

FC59-Development of a canine skin analog

Marques M.[1], Nunes J.[2], Ustymenko B. [1], Fialho L.[3,4], Martins L.[3,4], Burke A.[2,5], Souza E.F.[6], Filho C.M.C.[6], Craveiro A.C.[6], Costa A.R. [1,7], Branco S.[3,4], Antunes C.M.[1,7]

[1]Institute of Earth Sciences - ICT, [2]Department of Chemistry, School of Sciences and Technology, University of Évora, Portugal; [3]Mediterranean Institute for Agriculture, Environment and Development – MED; [4]Department of Veterinary Medicine, School of Sciences and Technology, University of Évora, Portugal; [5]Institute for Research and Advanced Learning – LAQV; [6]BRinova Biochemistry Lda., Évora, Portugal; [7]Department of Health and Medical Sciences, School of Health and Human Development, University of Évora, Portugal.

Email address: mrmarques@uevora.pt

Background. Skin covers an extensive area of the body and comprises three major layers, subcutis, dermis, and epidermis. This organ is one of the most tested for toxicity and safety evaluation during the process of drug research and development, in past usually performed *in vivo* using animals. On the last years a concern of sustainable and eco-friendly approach has been developing, with the expansion of non-animal alternatives (Klicks et al.; Mathes et al.).A histotypical cell cultured-derived tissue may be used to replace animal testing and are imperative to avoid armful, drawn-out tests to assess chemicals for their capacity to erode, bother or sensitize the skin. There are validated epidermal models for human (EPISKIN™ and EpiDerm™) and rat (TER) skin (Flaten et al.; NIEHS; Netzlaff et al.). The aim of our study was the development of a histotypical canine skin equivalent, that can be used for the assessment of corrosion, irritation and sensibilization, avoiding *in vivo* animal testing.

Methods. Canine keratinocyte progenitor cells were seeded in inserts and were allowed to grow until differentiation was reached, using an adapted version of the CELLnTEC commercial protocol, specific for human cells (CELLnTEC). For histological analysis, samples were fixed in 10% neutral-buffered formalin. Three-micrometer paraffin sections were routinely processed for biopsies and stained with hematoxylin and eosin. Corrosion, irritation and sensibilization protocols were adapted from human equivalent validated tests.

Results. A multilayer (3-4 cell layers thick) of canine keratinocytes was developed in air-lift culture, originating a stratified epidermal-like tissue, confirmed by histological analysis. This epidermal-like tissue exhibited functional characteristics of normal epidermis. It showed adequate impermeabilization, after 0.1% Triton X-100 exposure for 4h and responded adequately to the positive (5% SDS and glacial acetic acid) and negative (PBS) controls used in “in vitro” corrosion and irritation assessment.

Conclusions. As predicted, a canine skin analog was developed. This is a promising skin model for non-animal safety tests of veterinary pharmaceuticals or cosmetics, reducing in vivo testing, and can be commercialized as a service or a product.

References

CELLnTEC. *3D Keratinocyte Starter Ki, thttps://cellntec.com/wp-content/uploads/pdf/3D_Starter_Kit_HPEK.pdf, accessed August 13, 2021*

Flaten, Gøril Eide, et al. “In Vitro Skin Models as a Tool in Optimization of Drug Formulation.” *European Journal of Pharmaceutical Sciences*, vol. 75, 2015, pp. 10–24, doi:10.1016/j.ejps.2015.02.018.;

Klicks, Julia, et al. “In Vitro Skin Three-Dimensional Models and Their Applications.” *Journal of Cellular Biotechnology*, vol. 3, no. 1, 2017, pp. 21–39, doi:10.3233/jcb-179004.;

Mathes, Stephanie H., et al. “The Use of Skin Models in Drug Development.” *Advanced Drug Delivery Reviews*, vol. 69–70, Elsevier B.V., 2014, pp. 81–102, doi:10.1016/j.addr.2013.12.006.;

Netzlaff, Frank, et al. “The Human Epidermis Models EpiSkin®, SkinEthic® and EpiDerm®: An Evaluation of Morphology and Their Suitability for Testing Phototoxicity, Irritancy, Corrosivity, and Substance Transport.” *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 60, no. 2, 2005, pp. 167–78, doi:10.1016/j.ejpb.2005.03.004.;

NIEHS. *Episkin™, EpiDerm™, and Rat Skin Transcutaneous Electrical Resistance (TER) In Vitro Test Methods for Assessing the Dermal Corrosivity Potential of Chemicals*. 2001

Acknowledgments. Thiswork was co-funded by the Project NAQUIBIO DPSA - Obtenção de MedicamentosVeterinários a partir de Nanopartículas Prata fixadas em Bases Poliméricas deHidrogéis de Quitosana, Alentejo 2020, Co–Promotion, Ref. 03/SI/2017(ALT20-03-247-FEDER-033578) Co-Financed by FEDER Funds through the Operational Programme for Competitiveness Factors – COMPETE 2020 and by National Funds through FCT – Foundation for Science andTechnology under the Project UIDB/50006/2020, UIDB/04683/2020 and UIDP/04683/2020.

.

FC60-Development of chitosan-based nanocarriers with pharmacological activity for human skin wound healing - Optimization Method

Gastalho, C.M.[1], Filho, C. M.C.[2], Antunes, C. M.[1]

[1]Department of Health and Medical Sciences. School of Health and Human Development & Institute of Earth Sciences. University of Évora. Évora, Portugal; [2]BRinova Biochemistry Lda. Évora. Portugal.

Email address: cgastalho@gmail.com

Background & Aim. Both chronic and acute wounds are an health problem with huge impact on society and the World Health Organization describes them as the new hidden epidemic, affecting millions of people worldwide [1]. In Portugal, chronic wounds affect 3.3/1000 habitants and, besides human suffering, bringing high economic costs [2]. The aim of this project is to develop new ecologically and economically sustainable biotechnology-based nanocarriers containing pharmacologically and controlled-release multifunctional compounds incorporated and stabilized in hydrogels for accelerated skin wounds healing.

Methods. With improved adaptations, the ionic gelation method (IG) was chosen to synthesize either chitosan nanoparticles (NP-Ch) also chitosan nanoparticles entrapped with pharmacological compounds (NP-Ch(Opx-2) [3]. The Fourier Transform Infrared (FTIR) [4] was used for chemical characterization of the raw material and the obtained nanocomposites based on the returned transmittance spectra. The Dynamic Light Scattering (DLS) [5] was used to analyze nanoparticles size (nm), stability in suspension (PDI index) and Z-average (ζ) (intensity weighted mean hydrodynamic size of particles measured). The Scanning Electron Microscope (SEM) analysis prove about surface morphology nanoparticles and provide information relating to its three-dimensional structure.

Results. FTIR analysis allowed the chemical characterization transmittance spectra of the raw materials and the final nanocarriers. Peak characteristic of chitosan (3285,73 cm-1 , 2877,03 cm-1 , 11657,05 cm-1 , 1582,02 cm-1 , 1376,46 cm-1 and 1024,60 cm-1) was observed in the nanoparticles suspension. From DLS analysis we´ve obtained NP-Ch average size with 250,5±32,7nm, which represents 99,2±1,4% of these particles in the sample. Also, the PDI was 0,228±0,02 and ζ was 194,9±17,1 nm (d.). For the NPCh(Opx-2) two groups of particles were observed sized 346,5±130,3nm and 358,5±411,4nm, where the first is representative of 96% of the particles in the sample. The PDI was 0,628±0,104 and ζ was 562,7±180 nm (d.). The dispersed nanoparticles were detected by SEM, however morphological characterization was not possible.

Conclusions. The IG method proved to be reproducible and feasible in obtaining nanocompounds with desired sizes. The NP-Ch produced proved to be stable while the nanocompounds NP-Ch(Opx-2) revealed low PDI, which may be related to the dispersant used at the time of DLS analysis (water). The presence of the components in the nanosystems was confirmed by FTIR. Despite the difficulty to characterize the nanoparticles morphologically, the nanocompounds produced have the potential to be further developed and eventually used as wound healing materials.

References.

[1] A. Vaz, «Prevalência de Feridas num Distrito do Norte de Portugal», Universidade Católica Portuguesa, 2014.
[2] P. J. P. Alves, «Feridas: Prevalência E Custos», Universidade Católica Portuguesa, 2014.
[3] S. Pedroso-Santana e N. Fleitas-Salazar, «Ionotropic gelation method in the synthesis of nanoparticles/microparticles for biomedical purposes», Polym. Int., vol. 69, n. 5, pp. 443–447, 2020, doi: 10.1002/pi.5970.
[4] A. Dutta, «Fourier Transform Infrared Spectroscopy», Spectrosc. Methods Nanomater. Charact., vol. 2, pp. 73–93, Mai. 2017, doi: 10.1016/B978-0-323- 46140-5.00004-2.
[5] S. Bhattacharjee, «DLS and zeta potential – What they are and what they are not?», 2016, doi: 10.1016/j.jconrel.2016.06.017.

Acknowledgments. This work is supported by national funding awarded by FCT - Foundation for Science and Technology, I.P., projects UIDB/04683/2020 and UIDP/04683/2020 and by BRinova - Health and Innovation, Lda.

FC61-A graphical journey through the MBStox project

Marques-da-Silva D[1], Rodrigues JR[1] and Lagoa R[1]

[1]School of Technology and Management, Polytechnic Institute of Leiria, Portugal

Email address: dorinda.silva@ipleiria.pt

Polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) are widespread organic toxicants raising increasing concerns for their impact in the environment and human health. Moreover, skin exposure and absorption of pollutants is gaining attention for toxicological studies. The MBStox project funded by FCT (PTDC/BIA-MIB/31864/2017) is focused on the development of biosystems to face, at least in part, the concerns regarding PAHs and OPPs toxicology. The main directions are new methods of decontamination, protection and toxicological assessment.

The host institution of MBStox is the Polytechnic Institute of Leiria and the partner institutions are NOVAidFCT and Coimbra Chemistry Centre. Major aims include the development of: 1) materials for toxicants adsorption; 2) biocatalytic systems for toxicants degradation; 3) controlled release and skin absorption systems of flavonoids and toxic mixtures.

A pictorial representation of this multidisciplinary project was devised as a preview to capture the interest of the Congress participants. This poster will provide a run-through of the research activities and outputs of the project. While still undergoing, several developments were already accomplished, resulting in diverse publications and conference communications, and outreach interactions engaging novel audiences with technical and scientific issues. The project allowed the implementation of novel methods, including a work station specific for skin permeability studies and determination of permeation parameters using skin and other barriers. Materials were developed depending on the needs of each task including absorbent and biocatalytic systems for water decontamination of PAHs, biopolymer carriers and easy-to-use patches for skin application. In parallel, a science outreach activity enabled a closer contact with younger students, and a website constantly updated according to the project evolution was built (1).

In this poster, the audience will be conducted through the MBStox project via photographs taken in the laboratory, industry and schools, and schemes representing concepts worked out at the boundaries of Biochemistry with Bioengineering and Toxicology.

References.

1. MBStox website - https://mbstoxproject.wixsite.com/mbstox

FC62-Electrografted anthraquinone as a probe to measure pH changes at biofilm-anode interface in a wastewater operating microbial fuel cell

Nazua L. Costa[1], Germaine Olorounto[2], Thomas Flinois[2], Estelle Lebègue[3] and Frédéric Barrière[2]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República-EAN, 2780-157, Oeiras, Portugal; [2] Univ Rennes, CNRS, Institut des Sciences Chimiques de Rennes - UMR 6226, F-35000 Rennes, France; [3] Université de Nantes, CNRS, CEISAM UMR 6230, F-44000 Nantes, France.

Email address: nazuacosta@itqb.unl.pt

Background: Bioelectrochemical systems (BES) are gaining momentum as an alternative approach for the energy harvesting and biofuel production. However, the large-scale implementation of such BES is still far for a common reality, due to poor understanding of the mechanisms that rule the bacteria-electrode interface towards increased power outputs. For instance, changes of local pH usually led to alterations on the bioenergetics process of microbial communities and hence on bioelectrochemical devices performances, being thus an important biochemical process to be understood.

Method: Electrografted 1-aminoanthraquinone was used as a probe to monitor the pH changes at the biofilm-electrode interface on the anode of a microbial fuel cell inoculated with wastewater. This has been achieved by covalent grafting of 1-AAQ through electrochemical reduction of aryl-diazonium cations to its aryl radical.

Result: The variation of the formal potential of the grafted quinone as a function of pH was linear over the pH range 1 to 10 with a slope of -64 mV. This allowed to estimate an interfacial pH change of 0,8 pH unit, from 6.4 to 5.6, during 21 days of biofilm formation on the surface of the 1-AAQ-modified graphite electrode.

Conclusions: Electrografted anthraquinone yields a robust pH sensor with a large operational range. The modified electrode sustains electroactive biofilm development. Surface modification of graphite electrodes by quinone-like molecules can be a biocompatible method to monitor the interfacial pH during biofilm formation.

FC63-Ghost hunting: FhuF - a ferric-siderophore reductase of unknown structure

Trindade, I.B.[1], Hernandez, G. [1], Rollo, F. [1], Lebègue, E [2]., Barrière, F. [3], Vicente, J.B.[1], Moe, E[1]., Matias, P.M. [1,4], Cordeiro, T. [1], Piccioli, M. [5,6] & Louro, R.O[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Universidade Nova de Lisboa, Av. da República (EAN), 2780-157 Oeiras, Portugal, [2] Université de Nantes, CNRS, CEISAM UMR 6230, F-44000 Nantes, France, [3] Univ Rennes, CNRS, Institut des Sciences Chimiques de Rennes – UMR 6226, F-35000, Rennes, France, [4] Instituto de Biologia Experimental e Tecnológica (iBET), Apartado 12, 2780-901 Oeiras, Portugal, [5] Magnetic Resonance Center (CERM) and Department of Chemistry, University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy, [6] Consorzio Interuniversitario Risonanze Magnetiche Di Metallo Proteine (CIRMMP), Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy

Email address: ines.trindade@itqb.unl.pt

Iron is essential to virtually all forms of life. However, in the present oxygen-rich atmosphere, iron precipitates in its ferric form becoming not readily bioavailable. To circumvent this problem, microorganisms scavenge iron using small molecules called siderophores. These have some of the highest affinities for ferric iron, and thus, inside the cell iron release from these complexes does not occur spontaneously. Instead, this process is mediated by specific proteins that can be grouped in two families: the siderophore-interacting protein (SIP) family that have a flavin cofactor, and the ferric reductase family (FSR) characterized by proteins that contain a 2Fe-2S cluster. FhuF, from laboratory strain *Escherichia coli* K-12 is the only FSR ever isolated and it contains an atypical 2Fe-2S with the motif Cys-Cys-X_{10}-Cys-X_2-Cys. Although the function of FhuF as a ferric-siderophore reductase was already established, there is presently no knowledge regarding its structure and how this atypical 2Fe-2S cluster operates to mediate ferric-siderophore reduction. In this work, a combination of paramagnetic NMR spectroscopy, electrochemistry, far-UV circular dichroism (CD) and small angle X-ray scattering (SAXS) was used to investigate the structural and functional characteristics of FhuF from *E.coli* K-12.

FC64-Development of a LSPR-based biosensor for the SARS-CoV-2 detection

Moreira V.[1], Lapa H.[1,2], Celeste R. [3], Fantoni A.[1,4] Alegria E.[1,2], Matos A.P.A.[5], Almeida M.G.[5,6]

[1] ISEL-Instituto Politécnico de Lisboa, R. Conselheiro Emídio Navarro, n. 1, 1959-007 Lisboa, Portugal;
[2] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, [3]Sapienza University of Rome, Dep. of Chemistry and Drug Technologies, Piazzale Aldo Moro, 5, Roma, 00185, Italy.
[4] Centro de Tecnologia e Sistemas, UNINOVA, [5]Centro de investigação interdisciplinar Egas Moniz (CiiEM), Instituto Universitário Egas Moniz, Quinta da Granja, 2829-511, Caparica, Portugal.
[6] UCIBIO-REQUIMTE Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516, Monte de Caparica, Portugal.

Email address: mg.almeida@fct.unl.pt

Background. COVID-19 is an infectious disease caused by the SARS-CoV-2 virus that can cause mild to moderate or severe symptoms. Although, the implementation of vaccination programs had a positive impact in the pandemic management, we still need to maintain respiratory etiquette and physical distance to avoid the spread of the virus, and testing the population remains crucial. This project aims at developing of an easy-to-use, fast, and very sensitive antigen test to detect the SARS-CoV-2 virus, allowing quick isolation of positive cases. This new biosensor will combine the high specificity provided by monoclonal antibodies (Ab) targeting the Spike protein, with the high sensitivity of Local Surface Plasmonic Resonance (LSPR) optoelectronic transducers. To this end, the effective immobilization of the Ab on gold nanoparticles (AuNPs) is essential to maximize the LSPR effect and the binding of antigens. Herein, we will present the work done on the construction of the biosensing elements, i.e., the bionanoconjugates of gold nanoparticles with antibodies (AuNPs/Ab).

Methods. The AuNPs were synthesized by the Turkevich method[1] (15 nm) or purchased to Cytodiagnostics (15, 30, and 60 nm). Antibodies with different specificities for the antigen (Spike protein S1 and receptor binding domain) were from GenScript and R&D Systems. The AuNPs were functionalized with different capping agents (MUA, MBA or a combination of both), coupled to the cross-linkers EDC/NHS in different proportions. To find the optimal immobilization conditions, different AuNPs/Ab ratios, buffers, reaction times and centrifugation parameters were tested. The resulting bionanoconjugates were characterized by visible spectrophotometry using a customized portable spectrophotometer from Thorlab, agarose electrophoresis, and Transmission Electron Microscopy (TEM).

Results. The best results were obtained with the AuNPs prepared in-house. The surface coverage with MUA was more effective than with the other thiols. The immobilization of the antibody against the Spike protein was more effective, providing a slight shift in the plasmonic band upon antigen binding.

Conclusions. In this work we were able to prepare bionanoconjugates that are specific for the SARS-CoV-2 antigens in optimal conditions. The binding event can be monitored by UV-Vis spectrophotometry. Future work includes the development of the LSPR immunosensor through the immobilization of the bionanoconjugates onto the surface of a transparent substrate (Indium Titanium Oxide, ITO).

References.

1. Turkevich J, Stevenson PC, Hillier J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss Faraday Soc*. **1951**;11(c):55–75.

FC65-Inhibition of the Ca^{2+}-ATPase by Gold (I, III) Compounds

Custódia Fonseca[1], Gil Fraqueza[2], Sónia A.C. Carabineiro [3], Aureliano, M.[4*]

[1] CCMar, FCT, Universidade do Algarve, 8005-139 Faro, Portugal; [2]CCMar, ISE, Universidade do Algarve, 8005-139 Faro, Portugal; [3]LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal, sonia.carabineiro@fct.unl.pt
[4*]CCMar, FCT, Universidade do Algarve, 8005-139 Faro, Portugal.

Email address: maalves@ualg.pt

Gold compounds were used by our ancestors in the treatment of various diseases and today, they continue to be used in the treatment of rheumatism.[1] The study of inhibition of Ca^{2+}-ATPase by Au compounds were carried out for the first time and is reported here. Dichloro (2-pyridinecarboxylate) Au(III) (**1**); chlorotrimethylphosphine Au(I) (**2**); 1,3-bis(2,6-diisopropylphenyl) imidazole-2-ylidene Au(I) chloride (**3**) and chlorotriphenylphosphine Au(I) (**4**) were the compounds used and the IC_{50} values determined are 4.5 μM, 0.8 μM, 16.3 μM and 0.9 μM, respectively. The type of enzymatic inhibition, regarding to the native substrate MgATP, gold compounds **1** and **2** showing a non-competitive inhibition whereas for Au compounds **3** and **4** has a mixed type of inhibition. The results suggested that different compounds have different modes of interaction with the Ca^{2+}-ATPase, and Au(III) as Au(I) compounds showed good inhibitory capacity for this enzyme. Compounds **2** and **4** with IC_{50} < 1 μM is good indicative that Ca^{2+}-ATPase can be used as a therapeutic target in medicinal inorganic chemistry.[2]

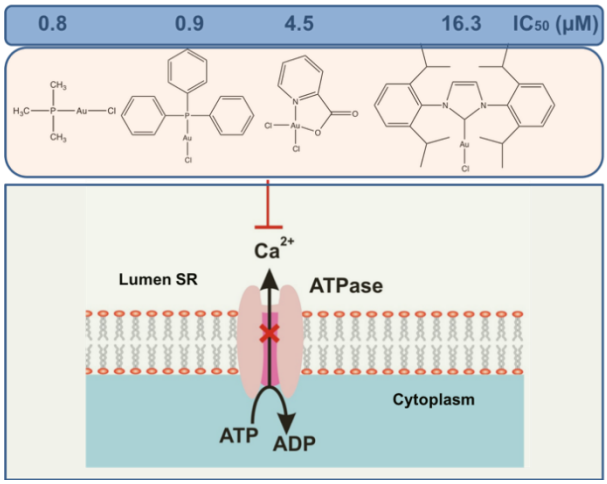


Figure 1. Gold compounds interacting with P-type ATPases, a putative target for gold anticancer activities (adapted from Figure 6 of Ref. 2).

References:

1. Higby, G.J. Gold in Medicine. *Gold. Bull* **1982**, *15*, 130–140.
2. Fonseca, C.; Fraqueza G.; Carabineiro, S.A.C.; Aureliano, M. *Inorganics*, **2020**, *8*, 49-

FC66-Bioactive peptides isolated from European amphibian skin secretions with possible anticancer effect

Pais do Amaral C[1], Plácido A [2], Teixeira C[2], Gomes P[2], Leite JRSA [3], Santos NC[1], Eaton P[2,4]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal; [2]LAQV/REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4051-401 Porto, Portugal; [3]Núcleo de Pesquisa em Morfologia e Imunologia Aplicada, NUPMIA, Faculdade de Medicina, Universidade de Brasília, 70910-900 Brasília-DF, Brasil; [4]The Bridge, School of Chemistry, Joseph Banks Laboratories, University of Lincoln, UK

Email address: maria.paisdoamaral@medicina.ulisboa.pt

Background. Bioactive peptides with potential therapeutic value have been isolated from amphibians for decades. They can exhibit many physiological activities such as antimicrobial, analgesic, antioxidant and anticancer (Xu and Lai, 2015). Four peptides were isolated from the skin of *Pelophylax perezi*, an amphibian captured in Azores islands (Portugal), and later also produced by chemical synthesis. One in particular, PpT-2, was found to belong to the tryptophyllin family (TPHs) (Chen et al. 2004), a heterogeneous small peptide group characterized by the presence of a tryptophan together with one or two proline residues. TPHs bioactivity remains uncertain, but their neuromodulator and antioxidant role were already studied. Indeed, some TPHs showed a sedative effect on birds, together with relaxation events in rat arterial and urinary bladder smooth muscle, as bradykinin antagonists (Wang et al. 2009). Since bradykinin is implicated in cancer progression, their antiproliferative effect in human prostate cancer cells was also tested and the proliferation inhibition was proven to be effective (Wang et al. 2013, 2014).

Methods. Therefore, in this work, we evaluated and further investigated the anticancer potential of PpT-2 and of three new histidine-rich peptides from *Pelophylax perezi* skin secretions resorting to viability assays.

Results. They displayed a moderate antiproliferative effect against prostate cancer cells.

References.

Xu, X.; Lai, R. The chemistry and biological activities of peptides from amphibian skin secretions. Chem. Rev. 2015, 115, 1760–1846.

Chen, T.; Orr, D.F.; O'Rourke, M.; McLynn, C.; Bjourson, A.J.; McClean, S.; Hirst, D.; Rao, P.; Shaw, C. Pachymedusa dacnicolor tryptophyllin-1: Structural characterization, pharmacological activity and cloning of precursor cDNA. *Regul. Pept.* 2004, *117*, 25–32.

Wang, L.; Zhou, M.; Chen, T.; Walker, B.; Shaw, C. PdT-2: A novel myotropic Type-2 tryptophyllin from the skin secretion of the Mexican giant leaf frog, Pachymedusa dacnicolor. Peptides 2009, 30, 1557–1561.

Wang, R.; Chen, T.; Zhou, M.; Wang, L.; Shaw, C. PsT-1: A new tryptophyllin peptide from the skin secretion of Waxy Monkey Leaf Frog, Phyllomedusa sauvagei. Regul. Pept. 2013, 184, 14–21.

Wang, R.; Lin, Y.; Chen, T.; Zhou, M.; Wang, L.; Shaw, C. Molecular cloning of a novel tryptophyllin peptide from the skin of the orange-legged monkey frog, phyllomedusa hypochondrialis. Chem. Biol. Drug Des. 2014, 83, 731–740.

Acknowledgments. This work was supported by FCT-MCTES fellowship PD/BD/136860/2018 and financed by national funds through the FCT— Fundação para a Ciência e a Tecnologia, I.P., within projects with references UIDB/50006/2020 and PTDC/BII-BIO/31158/2017. A. Plácido is a recipient of a post-doctoral grant from the latter project.

SPECIAL SESSION 1 – Art, Biocgemistry and Innovation in Life Sciences

In the last years Science and Art have been merging to create innovative views into the world. New paradigms where science, art and intervention strategies interlace fostering and innovation projects in:

1. Biochemistry goes to School: innovative projects for Biochemistry dissemination.
2. Education in Action: Biochemistry Olympiads and iDays;
3. Biochemistry and Society: Biochemistry applied to art and heritage.

Nos últimos nos a Arte e a Ciência têm emergido na criação de perpsctivas inovadoras sobre o Mundo. Novos paradigmas onde se interligam a Ciência, a Arte e as estratégias de intervenção social e projetos de inovação em:

1. A Bioquímica vai à Escola: projetos inovadores de divulgação da Bioquímica ao público;
2. Formação em ação: olimpíadas e IDays;
3. Bioquímica e Sociedade: a bioquímica dedicada à arte e ao património.

Biochemistry goes to School: innovative projects for Biochemistry dissemination

José Bragança	UALGORITMO, a new instrument for scientific outreach of the University of Algarve
Márcio Simão	Lab-it is taking molecular genetics to school

Education in Action: Biochemistry Olympiads and iDays

Renato Simões	Olimpíadas Universitárias da Bioquímica
GAITEC	IDays

Biochemistry and Society: Biochemistry applied to Art and Heritage

HERCULES Lab	Art & Biochemistry
Lab. de Antropologia Biológica	Let's get the bones "talking"!

Chairs: Leonor Cancela (UAlg), Manuel Aureliano (UAlg), Célia Antunes (UÉv)

Biochemistry goes to School: innovative projects for Biochemistry dissemination

UALGORITMO, a new instrument for scientific outreach of the University of Algarve

José Bragança

Faculdade de Medicina e Ciências Biomédicas, Algarve Biomedical Centre Research Institute – ABC-RI, Universidade do Algarve Campus de Gambelas, 8005-139 Faro Portugal

Email address: jebraganca@ualg.pt

Researchers at Universities generate and convey the knowledge acquired through communications in specialized (inter)national journals and congresses. Though, an effort to share the scientific achievements with the general public is extremely important. To this purpose, we have launched the UALGORITMO, a journal freely accessible online, in a downloadable PDF format, to disseminate the scientific activities of the University of Algarve. Articles submitted to the UALGORITMO are written in lay Portuguese language, by Researchers of the University, to summarise recent communications published by them in a peer reviewed journal. Master's and Doctoral students of the research groups are encouraged to contribute to the communications, even if they are not authors of the original publication, to participate in a publication for general public outreach and to train writing skills for concise communications. After submission, the manuscripts are revised by High Schools Students of the Algarve (10^{th} to 12^{th} year of scholarity), under the guidance of a school Teacher, for further simplification of the language used in the text and general improvement of the figures. After modifications of the manuscripts by the authors, based on the recommendations of the reviewers, and a final approval by the reviewers, the articles are edited and published in volumes containing 8 articles. The contribution of the reviewers is acknowledged at the end of each published article, by a concise description and a picture of the reviewers. In addition, the articles also include a summarised biography of the authors, and links to their research centres and teaching courses to increase the divulgation of the teaching activities of the University of Algarve. The participation of the Researchers and high school Students to the UALGORITMO has been very enthusiastic and dynamic, and has already allowed the publication three volumes of the UALGORITMO.

Biochemistry goes to School: innovative projects for Biochemistry dissemination

Lab-it is taking molecular genetics to school

Márcio Simão[1,2], Natércia Conceição[1,2], Susana Imaginário[3], João Amaro[4], M. Leonor Cancela[1,2]

[1]Comparative, Adaptive and Functional Skeletal Biology (BIOSKEL) lab, Centre of Marine Sciences (CCMAR), Universidade do Algarve, Faro, Portugal; *[2]Faculty of Medicine and Biomedical Sciences, Universidade do Algarve, Faro, Portugal*; *[3]CRIA (Divisão de Empreendedorismo e Transferência de Tecnologia), Universidade do Algarve, Faro, Portugal*; *[4] Innovatio sensum Consultoria Sociedade Unipessoal LDA*

The Molecular Genetics Moving Lab or “Laboratório itinerante de Genética Molecular” (Lab-it) was funded in 2008 by Leonor Cancela to promote the learning of molecular genetics introduced at that time into high school biology programs. The project aimed to introduce practical activities in molecular genetics to complement the theoretical concepts taught in school. These included the development of experimental protocols based on theoretical scenarios focusing on themes of forensics sciences, biomedical applications, diagnostic methods, and ecological research using basic molecular biology techniques such as DNA extraction, polymerase chain reaction (PCR), electrophoresis and restriction enzyme application. In these scenarios, the students execute all the procedures with the help of the Lab-it instructor and using the Lab-it equipment, followed by a discussion of the results with all the participants and the teacher. These approaches help the students to consolidate the concepts of molecular biology and simultaneously promote discussions on new advances in the area and choices for university careers. In addition to practical sessions, Lab-it also promotes seminars on topics of interest to the students and teachers. Since 2008 18 high schools have participated in the region of Algarve, averaging in each year about 400 students participating in practical activities. In 2021, despite the COVID pandemic, 9 schools and 379 students were involved in Lab-it practical sessions and 99% of them considered the activity to contribute to better understand the molecular biology methods approached in theoretical classes and expressed high interest on those sessions.

Education in Action: Biochemistry Olympiads and iDays

University Biochemistry Olimpiads

Renato Daniel

Universidade de Coimbra – Associação Académica de Coimbra

The University Biochemistry Olympiads are a pioneer educational project in the country, which aims to establish and recognize the academic merit of students. This project assesses the real skills of students on the national scene, independently of the socio-economic or academic conditions the student comes from. It is a pedagogical project that merely aims to assess the knowledge of higher education students, fostering a critical spirit regarding current problems, such as sustainability, technological transition or the economy, building bridges between these peripheral themes and Biochemistry itself, as a pillar structural science of the entire project. In a context where access to higher education is assumed to be limited for a large number of students, the Biochemistry Students' Nucleus of the Academic Association of Coimbra set the objective of carrying out this project in order to contribute to a fairer and more egalitarian higher education, ensuring continuity of studies to the winner, financed 1 year of tuition fees.

This is an ambitious, challenging and competitive project where it is possible to unite the Portuguese biochemical academic community, creating spaces for discussion and knowledge production, stimulating the Higher Education Institutions themselves to train more and better students, trained in the most varied areas. The University Biochemistry Olympiads are assumed not only as an important space for the exchange and sharing of ideas, but also as a space for valuing students, science and Biochemistry.

Education in Action: Biochemistry Olympiads and i-Days

EIT Health Innovation Days (i-Days): a student competition to tackle health challenges

Carlos Godinho[1], Filipe Loureiro[1], Hernâni Zão[1], Célia M. Antunes[2], Soumodip Sarkar[1,3]

[1]GAITEC - Divisão de Inovação, Cooperação, Empreendedorismo e Empregabilidade, Universidade de Évora;
[2]Departamento de Ciências Médicas e da Saúde, Escola de Saúde e Desenvolvimento Humano, Universidade de Évora;
[3]Departamento de Gestão, Escola de Ciências Sociais, Universidade de Évora; Évora, Portugal;

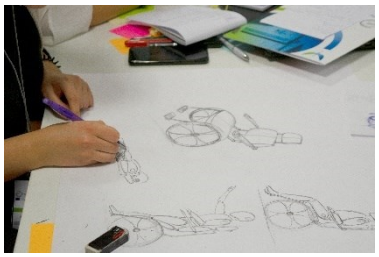
Innovation Days (i-Days) promote health innovation among university students through dozens of one-day and two-day programmes held in academic institutions around Europe. Students from all academic areas receive an introduction to practical health innovation tools and compete in teams to tackle real-life health challenges posed by EIT Health, local organisations, private corporations or start-ups.

Problems posed by health are complex, requiring capacity to deal with uncertainty and change; from a scientific and practical point of view, they cross disciplinary boundaries, and transdisciplinary skills are required which, based on collaboration and creativity to redefine and reframe them and design meaningful solutions, by integrating problem framing and problem solving, communication and collaboration between people of different disciplines and educational levels, and intelligent use of technologies and resources. By combining these competencies, it is intended to support the construction of collective knowledge and increase the capacity to solve human problems.

Health and Aging have been the focus of i-Days events at the UÉ and the challenges addressed the following problems: 2018 – Health and Aging: from the problem to solution; 2019 – Taking care home; 2020 – Post-pandemic Health Solutions: Responding to problems that appeared or aggravated with Covid19.

This year, new challenges will be addressed, will your solution win?

The winning team of each i-Day will attend the Winners' Event, a final competition that unites students from around Europe!



Ackowledgements: We thank NUDE - Núcleo Universitário Design de Évora for the images.

References:

Innovation Days, EIT Health, Europe. https://eithealth.eu/project/i-days/, viewed in Oct 30th, 2021;
Innovation Days, Universidade de Évora, Portugal. https://innovationday2018.xdi.uevora.pt/inovacao/, viewed in Oct 30th, 2021.

Biochemistry and Society: Biochemistry applied to Art and Heritage

Art & Biochemistry

Caldeira A.T., Martins M.R., Salvador C., Arantes S., Bhattacharya S., Palma V., Silva I.

Established in 2009, the HERCULES Lab is a research infrastructure from the University of Évora, devoted to the study and valorisation of cultural heritage, focusing on the integration of physical and material sciences methodologies and tools in interdisciplinary approaches.

Nowadays, the laboratory researchers are engaged in the material and historical study of different cultural heritage artifacts, namely, archaeological artifacts (ceramics, glass, metals, organic materials), art objects (easel paintings and polychrome sculpture, metals, historical textiles, ancient manuscripts), and built heritage (mortars, stone, mural paintings and glazed tiles).

Biochemical approaches are being used to study of the biodegradation processes of the materials used to produce artistic artifacts led to the development of novel biotechnology-based products used for identification of bio-contaminants and for materials conservation, that you can find in this exposition.

Let's get the bones “talking” !

Teresa Fernandes and Célia Lopes

Laboratory of Biological Antropology

Human skeletons constitute the most direct and trustworthy testimony of the populations that preceded us. In fact, they contain a great deal of information about the ways of life, the type of diet and the impact of pathogens and chronic diseases on the living and health patterns of populations in the past.

Today, the study of skeletons allows, after the usual macroscopic analyses, the use of molecular biology and biochemical techniques for a more detailed analysis. Examples of this action are the use of collagen to calculate isotopic ratios or the analysis of peptides, which are fundamental in the reconstitution of diets, or the use of ancient DNA for attributing the aetiology of some diseases.

The exhibit illustrates some of the ways in which neoplastic, metabolic, and infectious diseases can reach bone tissue.

FC67 - Microorganisms and Moonmilk in the non-ornated caves from the Vézère Valley (Dordogne, France)

Sriradha Bhattacharya[1,2], Ana Teresa Caldeira[2,3], Rémy Chapoulie[1], Catherine Ferrier[4], José Mirao[2,5], Delphine Lacanette[6], Léna Bassel[4], Catia Salvador[2]

[1] IRAMAT-CRP2A UMR 5060—CNRS-Université Bordeaux Montaigne, Maison de l'archéologie, Esplanade des Antilles F-33607, Pessac Cedex, France ; [2] HERCULES Laboratório, Universidade de Évora - Largo Marquês de Marialva, 8, 7000-809 Évora ; [3] Department of Chemistry, School of Science and Technology, Rua Romão Ramalho, nº 59 - Colégio Luís António Verney, 7000-671, Évora ; [4] PACEA UMR 5199—CNRS-Université de Bordeaux, Bâtiment B8, Allée Geoffroy Saint-Hilaire, CS 50023 F-33615 Pessac, Cedex, France ; [5] Department of Geosciences, School of Science and Technology, Rua Romão Ramalho, nº 59 - Colégio Luís António Verney, 7000-671, Évora ; [6] I2M UMR 5295—Bordeaux INP, Bordeaux F-33607 Pessac Cedex, France

Background. Microorganisms inhabit all possible environments including hypogean environments. Cave are the best examples of a glimpse into the subsurface world and into human past through its art work. Microbes are often harmful for cultural assets (eg, paleolithic paintings), because they are related to constructive (mineral precipitation) and destructive (substrate dissolution) processes affecting different substrates (host-rock, speleothems, paintings, etc.). Moonmilk, a secondary speleothem is a problem that plagues this art. The environment is very distinct as they differ vastly from the exterior owing to it being divided by the soil and the epikarst. Caves are considered as extreme environments due to very little or complete absence of sunlight and limited interaction with the outside ecosystem. This determines the growth of microorganisms that can easily adapt to these extreme conditions playing an important role in the development of biotransformations inside the caves, namely biomineralization and probably in the formation of moonmilk and leading to potential degradation of cave art. Moonmilk is identified by its distinctive crystalline fibre, referred to as Needle Fibre Calcite (NFC).

Methods. Moonmilk formation is not completely understood being attributed to abiotic processes and / or mediated by biotic processes. This study deals with the identification of the microorganisms sampled in three non-ornated caves (named Leye, Pillier and Racine) in the Vézère Valley (Dordogne, France). Leye is considered as a "laboratory cave", because it is very similar to Lascaux, located in the same region but without any cave art, mimicking the environmental conditions and making it an ideal cave to carry out multidisciplinary studies where sampling is allowed. The other two caves (Pillier and Racine) are also important because they show the same moonmilk presence and enable to check the variability of these bioinduced minerals. These two caves have not been submitted to any kind of study yet.

Results. *In situ* DinoLite Microscopy confirmed the existence of microorganisms and needles. *In vitro* culture showed the presence of bacteria, fungi and yeast. High Throughput Sequencing (HTS) was used to explore, compare and characterise the microbial communities present in the cave. Scanning Electron Microscopy helped us discriminating the different types of needles along with microbial deposits present in the caves. SEM micrographs show the presence of various needles: monocrystalline, polycrystalline and serrated, which occur due to biomineralisation. Bacterial communities are mainly composed by *Proteobacteria, Actinobacteria* and *Firmicutes.* Phylums like *Nitrospirae, Tenericutes, Spirochaetes* and *Verrucromicrobia* are also present in the caves.

Conclusions. Taking into account these data, the next step is to perform some simulation assays to better understand the microbial involvement for the growth of moonmilk.

Keywords: Moonmilk, Needle Fibre Calcite, prehistoric caves, Vézère Vallée, HTS, SEM imagery, conservation

SPECIAL SESSION 2 – COVID Special Session (PT)

The COVID-19 pandemic has challenged the community globally, profoundly defying our way of living.

The objective of this session is to present the characteristics of this pandemic and discuss the challenges facing government entities, institutions, the scientific community, and the public. It also intends to contribute to the construction of new perspectives, enhancing interdisciplinary approaches in the context of interventions in Public Health (press release at https://www.cm-evora.pt/sessao-especial-covid-19-deu-perspectiva-da-pandemia-em-evora/).

A pandemia COVID-19 desafiou a comunidade globalmente, afetando profundamente nosso modo de vida.

O objetivo desta sessão é apresentar as características desta pandemia e discutir os desafios que as entidades governamentais, instituições, a comunidade científica e o público em geral enfrentam. Pretende ainda contribuir para a construção de novas perspetivas, potenciando abordagens interdisciplinares no contexto das intervenções em Saúde Publica (nota de imprensa em https://www.cm-evora.pt/sessao-especial-covid-19-deu-perspectiva-da-pandemia-em-evora/).

*

Carlos Pinto Sá	Respostas e desafios sociais do município
Ana Costa Freitas	Respostas e desafios do Ensino Superior
Ricardo Mexia	“Desafios ao nível da Saúde Pública: aprendizagens e estratégias de prevenção”
Isabel Pita	“Desafios para a gestão de um Hospital Público/SNS em Pandemia”
Miguel Castanho	“Desafios de natureza molecular na compreensão da COVID-19: o papel da bioquímica presente e futuro”

Chairs: Victor Ramos (Diretor da ESDH, UÉv); Graça Soveral (FFUL, Presidente da SPB)

SUPLEMENTARY INFORMATION

1. Comunications presented in the congress

Registrations	163
Plenary lectures (50+10 min.), PL	4
Invited lectures (25+5 min.), IL	17
Oral communicatios (8+2 min.), OC	39
Flash communications (3+2 min.), FC	69
Total communications	129

2. Places from the speakers and type of communications presented

City/Country	Plenary	Invited	Oral	Flash	Total
Lisboa		1	14	16	31
Oeiras		1	6	14	21
Évora		1	4	12	16
Coimbra	1	2	3	6	13
Porto		2	6	4	12
Faro		2	1	7	10
Braga		1	2	3	6
Caparica	1	1	1	1	4
Badajoz/Spain		1		2	3
Germany	1	1			2
Leiria			1	1	2
Beja				2	2
Aveiro		1			1
Canada	1				1
Brasil		1			1
United Kingdon		1			1
Check Republic		1			1
Vila Real			1		1

3. COVID-19 Contingency Plan / Plano de Contingência COVID-19

All efforts are being made to ensure that the congress is held in person, hence the change of dates to 14th-16th October of 2021, when it is expected that the pandemic situation will be more controlled. We would like to point out that with this amendment, the deadlines for registration and submitting abstracts have also been extended. The registration, of those who have already registered, are obviously safeguarded.

Todos os esforços estão a ser envidados para que o congresso seja realizado no formato presencial, daí a mudança das datas para 14-16 de outubro de 2021, altura em que é espectável que a situação pandémica já esteja mais controlada. Chamamos à atenção de que com esta alteração foram também alargados os prazos de inscrição e de submissão de resumos. As inscrições, de quem já se inscreveu, estão obviamente salvaguardadas.

4. Registration and Submissions

The registration fee includes the congress materials, coffee-breaks, lunch (all registration type) and Congress Dinner (full time registration).

Fees	Senior		Junior (under 35y)		Student	
	SPB Member	**Non Member**	**SPB Member**	**Non Member**	**SPB Member**	**Non Member**
Early Fees **Until 9th of September**	180 €	250 €	100 €	130 €	65 €	100 €
Regular Fees **After 9th of September**	300 €		150 €		150 €	
One day registration Fees	50% the Regular fees					

SPB is awarding a limited number of grants (15). For more information, please see the SPB web page.

5. Registration procedure

1. Sign up in https://sge.uevora.pt (you will receive a confirmation email to finish your sign up)
2. After login in https://sge.uevora.pt go to "SPB 2020 XXI Nacional Congress of Biochemistry"
3. On the right side of the page choose "Register"
4. (Optional if you want to submit abstract) On the right side of the page choose "Submit Abstract"
5. After registration choose "Confirm Registration"
6. After you confirm your registration, you will be given the amount payable according to the options you selected in the option "Confirm payment".

6. Oral and Poster Guidelines

Oral Communications

Oral Communications have a 10 minute slot (8 minutes presentation + 2 minutes discussion).
Slides should be prepared with PowerPoint or Adobe Reader.
Official language for slides: English.
Official languages for oral exposition: English.
Speakers have to provide the presentation before the beginning of the corresponding session.

Poster Communications: Go digital! – ePoster

The XXI SPB National Congress of Biochemistry 2020 is environmentally and economically friendly!

The Posters will be digital (ePoster). Posters will be submitted in electronic format only (details will be made available in time) and the authors will have the opportunity to provide a short presentation (3 min) - Flash communications. The ePosters will be available to view throughout the Congress in eDesks and interaction with authors and viewers will be fostered.

Statistics

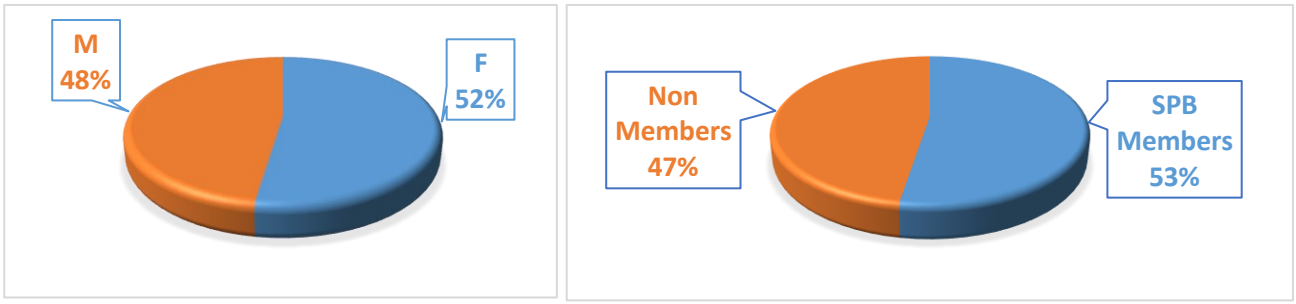


Figure 1. Participants distribution by sex (left) and according to SPB membership (right).

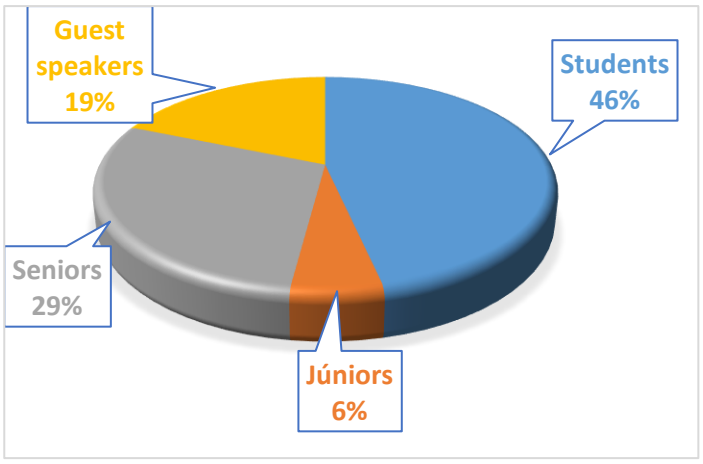


Figure 2. Participants distribution according to registration mode.

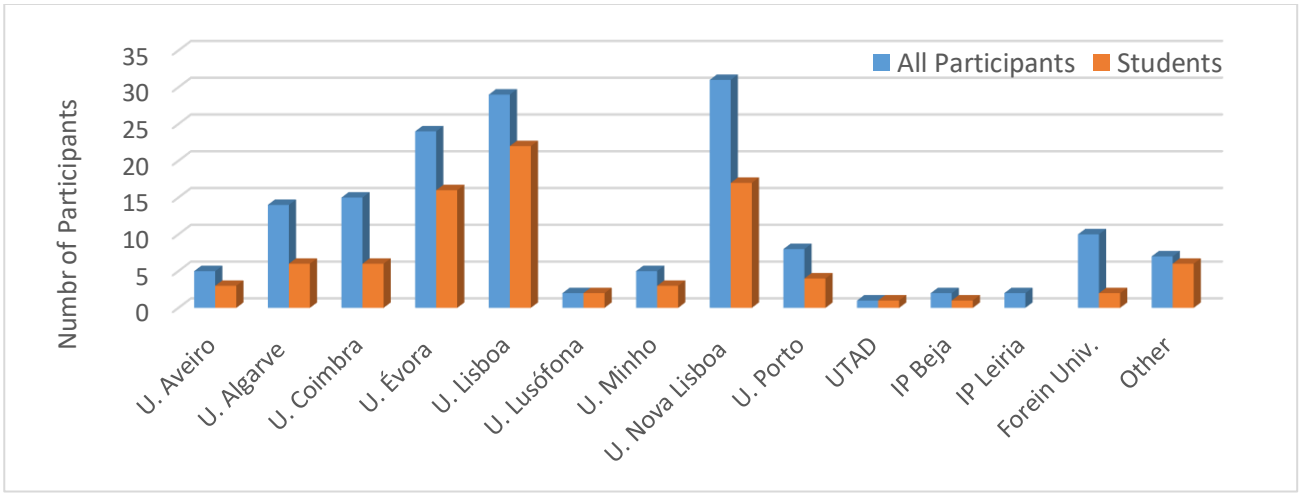


Figure 3. Distribution of Participants according to their institutions.

XXI SPB Congress Prize Awards

Awarded Oral communications

OS1a	**Inês V. da Silva**	Peroxiporins and pancreatic cancer: new roles for aquaporin-3 and aquaporin-5 in tumor biology
OS2	**Joana Figueiredo**	Inside proteomics: uncovering cultivar specific grapevine apoplast dynamics
OS3	**Patrícia Alves**	Effects of cannabidiol and delta-9-tetrahydrocannabinol on placental extravillous trophoblast cells migration
OS4/S7	**Henrique Fernandes**	The Nitrate and Nitrite Reductase Activity of Xanthine Oxidase: a Computational Study
OS1b/S5	**Margarida Saramago**	Targeting SARS-CoV-2 ribonucleases to combat COVID-19
OS8	**Ana Cláudia Leite** **Ricardo Soares**	Cell cycle-dependent regulation of ATP synthase beta subunit Exploration of the Desulfuromonadales “cytochromome”. Purification of multiheme cytochromes involved in the extracellular respiration of Desulfuromonas acetoxidans
OS9	**Ana Salomé Veiga**	Anti-HIV-1 activity and mode of action of pepRF1, a viral-derived CXCR4 antagonist

Awarded Flash/Poster communications

PS1a	**Bárbara Matos**	Chronic exercise training attenuates prostate cancer-induced molecular remodelling in the testis
PS2	**Ana Alhinho**	The dynamics of flower development in sweet chestnut tree (Castanea sativa mill.)
PS3	**Sander Noordam**	Photoelectroautotrophic growth of Rhodopseudomonas palustris TIE-1: The relationship between cytochrome c2 and the reaction center
PS4/S7	**Catarina Gonçalves Pimpão**	Unraveling the mechanism of aquaporin-10 irreversible inhibition by organogold compounds
PS1b/S5	**Beatriz Martins** **Daniela Alves**	Inflammation induces retinal pigment epithelium monolayer disruption and release of distinct populations of extracellular vesicles The role of understudied post-translational modifications on STAT3 behavior and function
PS6/S8/S9	**Patrícia Apura** **Ana Silva** **Inês B. Trindade**	A short trip to the world of Pseudomonas putida’s ribonucleases Cytochrome c maturation System III: can an eukaryotic system produce multiheme cytochromes? Ghost hunting: FhuF - a ferric-siderophore reductase of unknown structure

Previous SPB Congresses (1967-2021)

CONGRESSOS SPB

Congresso	Local	Ano	Organizador
I	Lisboa	1967	Gomes da Costa
II	Lisboa	1968	Gomes da Costa
III	Lisboa	1970	Gomes da Costa
IV	Lourenço Marques	1972	F. Carvalho Guerra
V	?	?	?
VI	Póvoa de Varzim	1978	F. Carvalho Guerra
VII	Póvoa de Varzim	1981	F. Carvalho Guerra
VIII	Póvoa de Varzim	1987	F. Carvalho Guerra
IX	Albufeira	1993	Winchil Vaz
X	Braga	1996	Cecilia Leão
XI	Tomar	1998	Arsélio P. Carvalho
XII	Póvoa de Varzim	2000	P. Moradas Ferreira
XIII	Lisboa	2002	M Manuela Chaves
XIV	Vilamoura	2004	Leonor Cancela
XV	Aveiro	2006	Manuel Santos
XVI	Ponta Delgada	2008	Maria L. Pavão
XVII	Porto	2010	Natércia Teixeira
XVIII	Coimbra	2014	Paulo J. Oliveira
XIX	Guimarães	2016	Margarida Casal
XX	Lisboa	2018	João Laranjinha
XXI	Évora	2021	Célia Antunes

SPB – from the Past to the Future

Biochemistry: the present comes from the future!

A Sociedade Portuguesa de Bioquímica (SPB) foi fundada em 1957 como uma secção independente da Sociedade de Ciências Médicas de Lisboa. Tornou-se uma sociedade autónoma em 1967, na sequência da introdução da disciplina de BIOQUÍMICA em curricula de diversos cursos de Universidades Portuguesas. Fundada oficialmente em 1975 por um grupo de prestigiosos médicos e professores universitários (DR III série, 183, 10.8.1989, 14098-14100).

Earlier times

SPB Clinical Biochemistry Worshops

Workshop	Local	Ano	Tema	Coordenador
I	Porto	2003	Patologias Inflamatórias	Natércia Teixeira
II	Porto	2006	Sinalização e doença	Natércia Teixeira
III	Porto	2008	Doenças emergentes do séc. XXI	Natércia Teixeira
IV	Faro	2010	Interactions between biochemistry and clinical pratice	Manuel Aureliano
V	Coimbra	2012	Translational molecular biochemistry	Catarina Oliveira / M. Grazina
VI	Lisboa	2014	Peroxissomes and mitochondria	Isabel Almeida
VII	Porto	2016	Obesidade: Da vida in útero à terceira idade	Natércia Teixeira
VIII	Évora	2018	Allergy and environment	Célia Antunes
IX	?	?	?	?

Education in Biochemistry in Portugal

Institution	City	Year	Poject (ECTS)	Options	Actual Director (BSc) *[8]	MSc
Universidade de Coimbra	Coimbra	1980	12	6*[1]	Luís Martinho do Rosário lrosario@uc.pt	Biochemistry
Universidade do Porto	Porto	1981	18	2	Paula Gameiro agsantos@fc.up.pt	Biochemistry
Universidade de Lisboa	Lisboa	1981	0	3	António Ferreira aeferreira@fc.ul.pt	Biochemistry
Universidade do Algarve	Faro	1993	0 or 12*[2]	4 or 2*[3]	Manuel Aureliano maalves@ualg.pt	Cellular and Molecular Biochemistry (2004)*[4]
Universidade da Beira Interior	Covilhã	1997	6	1	Renato Boto rboto@ubi.pt	Biochemistry
Universidade de Évora	Évora	1998	15	4	António Canto ammc@uevora.pt	Biochemistry
Universidade Nova de Lisboa	Caparica	2004	15	5*[5]	Cristina Costa mcoc@fct.unl.pt	1.Biochemistry (2012) 2.Biochemistry and Health (2013) *[6]
Universidade de Trás-os-Montes e Alto Douro	Vila Real	2005	12	2	Lucinda Reis lvrfisco@utad.pt	Biochemistry
Universidade de Aveiro	Aveiro	2006	14	1	Manuel António Silva mac@ua.pt	Biochemistry*[7]
Universidade do Minho	Braga	2007	15	4	Célia Pais cpais@bio.uminho.pt	Applied Biochemistry
Universidade da Madeira	Funchal	2007	7.5	4	Paula Castilho pcastilho@staff.uma.pt	Applied Biochemistry

*[1] 6 electives, 6 ECTS each;
*[2]If 2 optional disciplines chosen,
*[3]If project chosen.
*[4] Master was not yet open for the students.
*[5] 2 options 3 ECTs and 3 options 6 ECTS,
*[6]Master in Association between 3 Organizational Units of UNL, FCT, ITQB and FCM.
*[7] With 3 domains of specialization: Biomolecular methods, Food Biochemistry, Clinical Biochemistry.
*[8] Eventually, actualization of the Director might have occurred.

XXI SPB Congress Photo Gallery

October 14th



Welcome reception


October 15th


Pollen sampling
wind vane
Suction pump depends on electric
It allows the hourly analysis
UNIVERSIDADE DE ÉVORA


A graphical journey through the MBStox project
FCT


STAFF
STAFF
STAFF

Lunch time

Congress Dinner

October 16th

COVID-19 Special Session

Our Staff

PARTICIPANTS LIST

A

Abranches R.

Alhinho A.

Alves A.

Alves D. M.

Alves J.

Alves P.

Amaral C. P.

Ambrósio F.

Andrade J.

Antunes C.

Apura P.

Araújo Conceição T.

Arraiano C. M.

Aureliano, M.

B

Barros D.

Bhattacharya S.

Bragança J.

Branco A.

Brito M.

C

Cancela L.

Carmo-Silva E.

Carrageta D.

Carreira L.

Carrilho J.

Carrillo M. B.

Carvalho A. L.

Carvalho H.

Casal M.

Castanho M.

Castelo-Branco M.

Catarino A.

Cavaco A. R.

Coelho A.

Coelho P.

Corda P.

Correia P.

Cardoso H. G.

Costa A. R.

Costa S.

Costa N. L.

Costa M. N.

Costa V.

Cruz-Silva A.

Cucchiarini M.

Conceição T. A.

Custódio M.

D

Diallo M.

Dias C.

N

Neves V.

Noordam S.

Nunes J.

O

Oliva A.

Oliveira M.

Oliveira T.

P

Palma P.

Pastorinho M. R. D.

Penha M. A.

Peralta

Pimpão C. G.

Pimpão J. R. F.

Pinto F. R.

Poejo J.

Poudel S.

Q

Quadros M.

Quendera A.

Quintal J.

R

Ramalho-Santos J.

T

Tavares A.

Teixeira M.

Rego A. C.

Rendulic T.

Rocha B.

S

Santos J.

Santos M.

Santos N.

Santos-Pereira C.

Saramago M.

Sarmento M.

Seixas A.

Silva D. M.

Silva J. M.

Silva N.

Silva A.

Silva A.

Silva A.

Silva I. V.

Silvério M.

Simão M.

Simões R.

Soares C.

Soares R.

Sousa I.

Sousa A. C.

Sousa E.

Sousa J. R.

Soveral G.

Teixeira N.

Tendeiro A.

Trindade I. B.

V

Z

"Évora Face Element in the Street"
This iconic element is found in Rua da República. Autoria José Moura (October 2021)