7th ITQB NOVA PhD Students' Meeting

23rd-25th November 2016

Book of Abstracts

#itqbphdmeeting

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Letter of Welcome

Dear Colleagues,

We welcome you to the 7th ITQB NOVA PhD Students´ Meeting, which will be held between the 23rd-25th of November 2016.

The 3-day meeting provides, each year, an invaluable opportunity for ITQB NOVA PhD students to present and discuss their work amongst each other, but also with the entire ITQB NOVA scientific community.

This year, around 60 students will be presenting their work. The several oral and poster presentations will walk you through a broad spectrum of scientific areas – from Technology, Chemistry, Biological Chemistry and Biology to Plant Sciences.

In addition, do not miss our fine selection of invited speakers. They are experts in different fields of science, such as anthropology, medicine, astrobiology, and entrepreneurship. All of them will surely inspire and motivate us to move forward with our research and careers. The invited speakers of this year are: Eugénia Cunha, Thomas Hänscheid, Zita Martins and David Cristina.

The Organizing Committee wishes you a great 7th ITQB NOVA PhD Students´ Meeting.

The Organizing Committee of the 7th ITQB NOVA PhD Students´ Meeting,

Ana Fernandes
Ana Oliveira
Ana Rita Narciso
Ana Silva
Andreia Tavares
Aristides Mendes
Cátia Silva
Celso Martins
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Supports

The Organizing Committee is grateful for the generosity of the listed partners that made the 7th ITQB NOVA PhD Students´ Meeting possible.

Programme at a Glance

	Day 1 - Wednesday 23rd November		Day 2 - Thursday 24th November		Day 3 - Friday 25th November	
9:40 - 10:00	Registration		Session 2	Alicja Gorska	Session 4	Saúl Silva
10:00 - 10:20				Nazua Costa		Inês Figueira
10:20 - 10:40				Patricia Borges		Joana Santos
10:40 - 11:10				Coffee Break		Coffee Break
11:10 - 11:30				Sónia Neto		Catarina Esteves
11:30 - 12:00			Eugénia Cunha		David Cristina	
12:00 - 12:30						
12:30 - 13:00			Lunch		Lunch	
13:00 - 14:00	Lunch					
14:00 - 14:20			Thomas Hanscheid		Session 5	Ana Pereira
14:20 - 14:40	Opening Session					Carmen Santos
14:40 - 15:00	Session 1	Cátia do Carmo				Hugo Almeida
15:00 - 15:20		Cláudia Mourato	Session 3	Helena Sapeta		João Vidigal
15:20 - 15:40		Elsa Mecha		Margarida Rosa		Coffee Break
15:40 - 16:00		Coffee Break		Coffee Break		Mafalda Dias
16:00 - 16:20		Miguel Guerreiro		Mariana Palma	Zita Martins	
16:20 - 16:40		Rita B. Santos		Nuno Gonçalves		
16:40 - 17:00		Tiago Duarte		Soraia Caetano		
17:00 - 17:40	Poster Session 1 (Wine&Cheese)		Poster Session 2 (Wine&Cheese)		Poster Social Gathering	
17:40 - 18:30						
20:00					Dinner	

Session 1	Optimizing organisms performance
Session 2	From structure to mechanism
Session 3	Stress responses
Session 4	Enhancing diagnostic and therapeutics
Session 5	Pathogenesis and treatment strategies

Scientific Programme

Day 1 – Wednesday (23rd November)

9:00-14:00 – Registration

14:20 – Opening Session (ITQB Direction Member)

Session 1 – Optimizing organisms performance

Chairs: Nuno Almeida and Diego Hartmann

14:40-15:00 – O1 "Formulation of pea protein for increased satiety and improved foaming properties"

Cátia S. Carmo, Ana N. Nunes, Inês Silva, Catarina Maia, Joana Poejo, Susana Ferreira-Dias, Isabel Nogueira, Rosário Bronze and Catarina M. M. Duarte

15:00-15:20 – O2 "Biocatalytic interconversion of H_2 and formate"
Cláudia Mourato, Mónica Martins and Inês A.C. Pereira

15:20-15:40 – O3 "Assessing antinutritional compounds and protein quality of different Portuguese common bean (*Phaseolus vulgaris L.*) varieties"
Elsa Mecha, Maria Eduardo Figueira, Maria Carlota Vaz Patto and Maria do Rosário Bronze

15:40-16:00 – Coffee Break

16:00-16:20 – O4 "Cell-based biosensors for detection and quantification of label-free virus for research and diagnostics"
Miguel Ricardo Guerreiro, Ana Filipa Rodrigues, Paula Marques Alves and Ana Sofia Coroadinha

16:20-16:40 – O5 "Tailor-made plant cell cultures for optimized production of recombinant proteins"
Rita B. Santos, Andreas Schiermeyer and Rita Abranches

16:40-17:00 – O6 "Metabolic fingerprint of mAb-producing GS-CHO cells using labelled tracers"
Tiago M. Duarte, Reza Seifar, Aljoscha Wahl, Paula M. Alves and Ana P. Teixeira

17:00-18:30 – Poster Session 1 (Odd numbers) and Wine&Cheese

Day 2 – Thursday (24th November)

Session 2 – From structure to mechanism

Chairs: João Vicente and Ana Oliveira

9:40-10:00 – O7 "Identification and functional characterization of transcription factors regulating *ZmPEPC1* in maize"
Alicja Górska, Paulo Gouveia, Ana Rita Borba, Anna Zimmerman, Tânia S. Serra, M. Margarida Oliveira, Christoph Peterhaensel and Nelson J.M. Saibo

10:00-10:20 – O8 "Unraveling the mechanisms of extracellular respiration of thermophilic Gram-positive bacteria"
Nazua L. Costa, Hans K. Carlson, John D. Coates, Ricardo O. Louro and Catarina M. Paquete

10:20-10:40 – O9 "Flavodiirion proteins: a structural approach to understand the substrate selectivity"

Patrícia T. Borges, Célia V. Romão, Miguel Teixeira and Carlos Frazão

10:40-11:00 – Coffee Break

11:00-11:20 – O10 "Unravelling the molecular mechanisms that orchestrate the electron transfer from inside *Shewanella oneidensis* cells to terminal reductase cytochromes"
Sónia E. Neto, Catarina M. Paquete and Ricardo O. Louro[1]

11:30-12:30 – Invited Speaker: Eugénia Cunha – "How does Forensic Anthropology face the new challenges of nowadays societies: from terrorist attacks to crimes against humanity"

12:30-14:00 – Lunch

14:00-15:00 - Invited Speaker: Thomas Hänscheid – "Do you believe in magic? Homeopathy, acupuncture and other alternative treatments"

Session 3 – Stress Responses

Chairs: Ambre Jousselin and Miguel Costa

15:00-15:20 – O11 "Drought and subsequent recovery behaviour of the biodiesel plant *Jatropha curcas*: Integrating transcriptomics and physiology"
Helena Sapeta, Tiago Lourenço, Stefan Lorenz, Christian Grumaz, Philipp Kirstahler, Pedro M. Barros, Joaquim Miguel Costa, Kai Sohn and M. Margarida Oliveira

15:20-15:40 – O12 "Unveiling the key players of the rice SUMOylation machinery in drought response"
Margarida T. G. Rosa, Diego M. Almeida, Inês S. Pires, Daniel da Rosa Farias, Cecília Almadanim, Luciano Carlos da Maia, António Costa de Oliveira, Nelson M. J. Saibo, M. Margarida Oliveira and Isabel A. Abreu

15:40-16:00 – Coffee Break

16:00-16:20 – O13 "Study of seasonal weight loss tolerance in small ruminants – an NMR-Metabolomics approach"
Mariana Palma, André Martinho de Almeida and Manolis Matzapetakis

16:20-16:40 – O14 "Wolverine and SLR1, the only DELLA protein in rice: Separated At Birth"
Nuno Gonçalves, Cátia Nunes, André Cordeiro, Mafalda Rodrigues, M. Margarida Oliveira and Isabel Abreu

16:40-17:00 – O15 "Transcriptional and post-transcriptional mechanisms underlying cadmium detoxification in yeast"
Soraia M. Caetano, Catarina Pimentel and Claudina Rodrigues-Pousada

17:00-18:30 – Poster Session 2 (Even numbers) and Wine&Cheese

Day 3 – Friday (25th November)

Session 4 – Enhancing diagnosis and therapeutics

Chairs: Naiara Fernández and Joana Pais

9:40-10:00 – O16 "Novel synthesis of Oseltamivir and Tamiphosphor and other synthetic methodologies"
Saúl Silva and Christopher D. Maycock

10:00-10:20 – O17 "Crossing the blood-brain barrier: effects of human bioavailable (poly)phenol metabolites in neuronal cells"
Inês Figueira, Gonçalo Garcia, Rui Carlos Pimpão, Inês Costa, Lucélia Tavares, Filipa Almeida, M. Rita Ventura, Ana Paula Terrasso, Catarina Brito, Gordon McDougall, Derek Stewart, Dora Brites, Maria Alexandra Brito and Cláudia Nunes dos Santos

10:20-10:40 – O18 "Single N-terminal phosphorylation modulates mutant huntingtin aggregation and toxicity"
Joana Branco-Santos, Gonçalo M. Poças, Yolanda Pires-Afonso, Flaviano Giorgini, Pedro M. Domingos, Federico Herrera and Tiago Fleming Outeiro

10:40-11:00 – Coffee Break

11:00-11:20 – O19 "Colorimetric detection of pyrophosphate in water with dinuclear zinc(II) complexes of *m*-xylyl-bis-cylen"
Catarina V. Esteves, Raphaël Tripier and Rita Delgado

11:30-12:30 – Invited Speaker: David Cristina "From bench to Venture Capital"

12:30-14:00 – Lunch

Session 5 – Pathogenesis and treatment strategies

Chairs: José Andrade and Daniel Simão

14:00-14:20 – O20 "Staying in shape: *Staphylococcus aureus* FtsZ-dependent morphogenesis of elongated cells"
Ana R. Pereira, Jen Hsin, Ewa Król, Andreia C. Tavares, Pierre Flores, Egbert Hoiczyk, Natalie Ng, Alex Dajkovic, Michael S. VanNieuwenhze, Yves V. Brun, Terry Roemer, Rut Carballido-Lopez, Dirk-Jan Scheffers, Kerwyn Casey Huang and Mariana G. de Pinho

14:20-14:40 – O21 "Genomic approaches to understand the responses of *Castanea* spp. to infection by *Phytophthora cinnamomi* Rands"
Carmen Santos, Helena Machado, Susana Serrazina, C. Dana Nelson, Pedro Fevereiro and Rita Costa

14:40-15:00 – O22 "Treatment of aqueous effluents contaminated with active pharmaceutical ingredient (APIs)"
Hugo F. D. Almeida, Mara G. Freire and Isabel M. Marrucho

15:00-15:20 – O23 "Insect cells platforms for fast production of pseudo-typed VLPS for drug and vaccine development"
João Vidigal, Bárbara Fernandes, Mafalda M. Dias, Marco Patrone, Manuel J.T. Carrondo, Ana P. Teixeira and Paula M. Alves

15:20-15:40 – Coffee Break

15:40-16:00 – O24 "Accelerating Recombinant Protein Availability in Stable Insect Cells Through a Novel Recombinase-based Targeting Strategy
Mafalda M. Dias, João Vidigal, Marco Patrone, Manuel J.T. Carrondo, Paula M. Alves, António Roldão and Ana P. Teixeira

16:00-17:00 – Invited Speaker: Zita Martins – "Origin and detection of life in our solar system"

17:00-18:30 – Poster Social Gathering

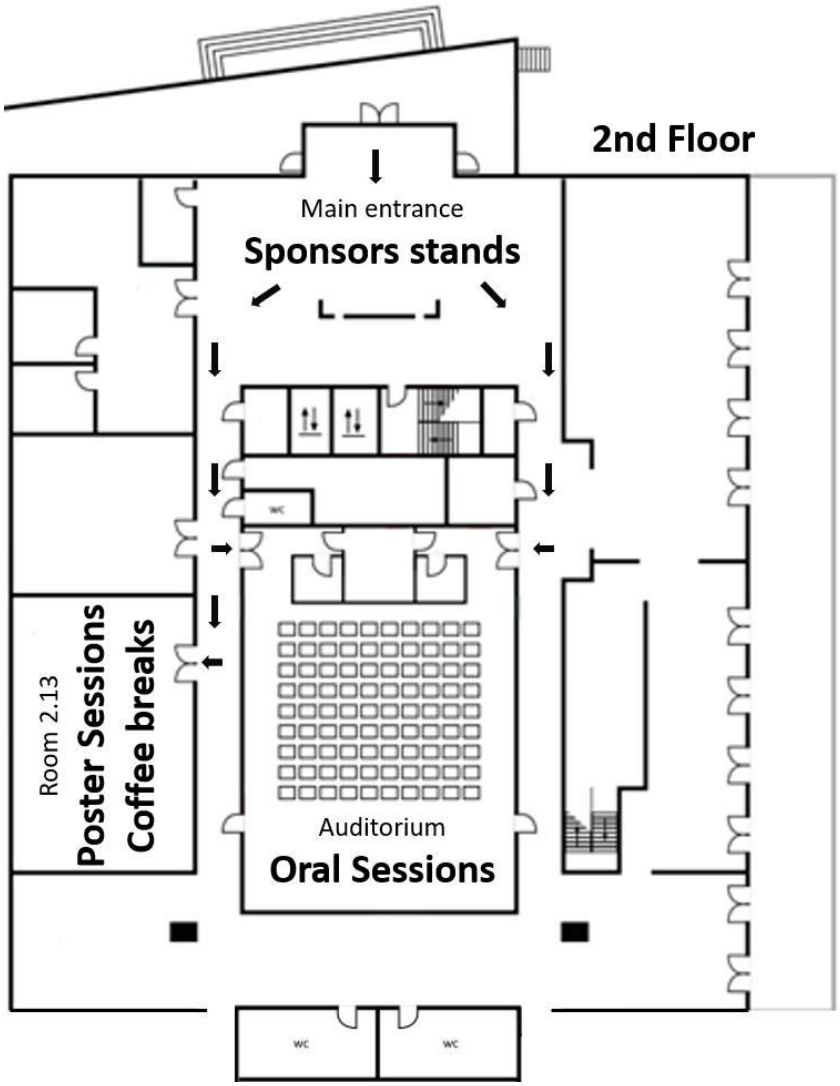
20:00 - Dinner

Venue Information

The 7th ITQB NOVA PhD Students´ Meeting will be held at ITQB NOVA's second floor. A quick look at the following map will show you where you can find the different activities.

Please note that the Invited speaker's talks will be held in the Auditorium.

The Meeting's Dinner will take place at Caravela D'Ouro, in Algés. During dinner, best oral and poster presentations will be awarded. In addition, our contributions will be donated to CERCIOEIRAS. The dinner is sponsored by Câmara Municipal de Oeiras.



INVITED SPEAKERS

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EUGÉNIA CUNHA

Full Professor

Universidade de Coimbra, Portugal

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[Photo by Daniel Rocha]

Eugénia Cunha has a PhD in Physical Anthropology (University of Coimbra) and since 2003 is a full professor at the University of Coimbra. She is the founder and the coordinator of the Forensic Anthropology Laboratory of University of Coimbra (http://lfa.uc.pt).

She acts as a National Consultant in Forensic Anthropology for the National Institute of Legal Medicine and Forensic Sciences in Portugal since 1997 where she became the forensic anthropologist of the South Delegation in 2004.

Fellow of the American Academy of Forensic Sciences. Co-Founder and President of FASE- Forensic Anthropology Society of Europe, 2009-2015. In 2014 she was certified as a C-FASE- *Honoris Causa* in Forensic Anthropology by FASE/IALM, International Academy of Legal Medicine. Founder member and scientific consultant of ABRAF- *Associação Brasileira de Antropologia Forense* (Brazilian Association of Forensic Anthropology), since 2014. Became a member of Pathology and Anthropology Sub-group, Interpol DVI Working Group, in 2016.

Eugénia Cunha has 31 years of experience with human bones and her field and laboratorial work is developed in several countries. Coordinated the 5 forensic anthropology missions to Guinea-Bissau, 2008-2010, to exhume and identify the Portuguese soldiers who died in the colonial war during the sixties and seventies. She was the forensic anthropologist of the mission to retrieve the body of a Portuguese military from a mass grave in East Timor (2003). In Mali, December 2013 –May 2014, she was the forensic anthropologist for JRR- Justice for Rapid Response.

How does Forensic Anthropology face the new challenges of nowadays societies: from terrorist attacks to crimes against humanity

Crimes against humanity and mass disasters had always been one of the key areas of expertise of forensic anthropology. Yet, in nowadays society, different types of mass disasters and new situations are requesting new methodological tools to face them. This is the case of the refugee crisis, leading to an increasing need to identify both living and dead migrants. It is also the situation of terrorist attacks where identification has sometimes to be done on the basis of fragments and body parts. On the other hand, crimes against humanity continue to happen almost everywhere. In this presentation we will approach how forensic anthropology can contribute to some of these cases, namely the identification of burned remains, fragments and body parts. Furthermore an example will be given in which this science was able to document violation of human rights. We argue that forensic sciences play a key role in the globalized societies of nowadays.

THOMAS HÄNSCHEID

Physician

Assistant Professor, Instituto de Microbiologia,

Faculdade de Medicina de Lisboa

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Thomas Hänscheid is Professor of Microbiology at the Instituto de Microbiologia, Faculdade de Medicina de Lisboa. He also worked as Clinical Microbiologist at the Hospital de Santa Maria up to 2010. Professor Hänscheid trained in medicine (Germany) and worked for 8 years as a clinician in internal medicine/infectious diseases (HIV/TB) in various Hospitals in London. He specialized in Clinical Pathology in Lisbon, Portugal with a focus on clinical microbiology. His research interests include the development of new diagnostic tests for malaria, schistosomiase and bacterial diseases (focus on tuberculosis (TB)) based on low cost fluorescent microscopy and flow/image cytometry, as well as the immune-pathogenitic role of the malaria pigment. He has also been working on interactions of pathogens, especially malaria and TB, malaria and other respiratory pathogens, malaria and non-typhoidal *Salmonella*. He has close collaborations with African Institutions especially with the Medical Research Unit of the Albert Schweitzer Hospital, Lambaréné, Gabon, which focuses on clinical research, with a particular interest in malaria and TB.

Do you believe in magic?

Homeopathy, acupuncture and other alternative treatments

"The desire to take medicine is perhaps the greatest feature which distinguishes man from animals."

(Sir William Osler)

Alternative medicine and alternative treatments are in vogue. In the UK or USA, up to 50% of people have tried it on themselves or their children. Portugal appears to be following suit. Homeopathic treatments, including the omnipresent Oscillococcinum for the winter flu; acupuncture, with primetime appearances of their practitioners on national TV shows; pills for memory, perceived lack of calcium or a better sex live; or even more "sophisticated" approaches, like quantum-medicine, electro-therapy, or homotoxicology; a myriad of diets to "detoxify" the body (or parts of it); topped by conspiracy theories why it would be better not to vaccinate your child to protect it from autism. The list of diseases which are "successfully treated" or even "cured" is long; ranging from more banal infections, back-pain, allergies, to live-threating diseases such as cancer or HIV.

What actually is alternative medicine? How does it work? Does it work at all? What are the concepts and theories behind it? How do these theories fit into science? How can I tell if an alternative treatment works or not? And anyway, are there not enough people who have experienced for themselves that it works? What could explain the true effectiveness of these therapies? Could that not be used in medicine? What is the impact of all this on the society's way of scientific reasoning and scientific culture in general?

This talk will address these issues in an engaging, provocative and hopefully, entertaining way.

DAVID CRISTINA

Senior Investment Consultant

Portugal Ventures

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David completed his Licenciatura (B.Sc.) in Microbiology and Genetics at Universidade de Lisboa and received a Ph.D. in Genetics of Aging from work he developed at the University of California, San Francisco.
From 2009 onward David spearheaded Instituto Gulbenkian de Ciência's Technology Transfer Office.
In 2011, he went on to found the biotech startup Acellera Therapeutics out of research developed at Instituto de Medicina Molecular, which received several awards (Idea2Product, Building Global Innovators, BES-Inovação e ANJE Jovem Empreendedor) and went on to raise private investment and public funding. David also founded the consulting firm Triple Helix Technologies focused on non-dilutive funding opportunities for research institutions and companies.
In 2012, David was invited to join the Cabinet of the Secretary of State for Entrepreneurship and Innovation as a Member (Adjunto), and later joined the Cabinet of the Secretary of State for Science with his role focused on public policies to maximize economic impact of R&D.
In 2015, he joined the Venture Capital Fund Portugal Ventures as part of their Life Science and Medtech investment team. David currently sits on the board of the startups Lymphact, Immunethep and Biotrend.

From bench to Venture Capital

A short presentation on the twists and turns of a career path leading from researcher to venture capitalist. Also, some insights on bringing academic discoveries to market, from the Venture Capitalist's perspective.

ZITA MARTINS
Royal Society University Research Fellow
Imperial College London
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Zita Martins is a Royal Society University Research Fellow at Imperial College London, working in the field of Astrobiology. Her research interests include the detection of bio-signatures in space missions, and the possible contribution of organic compounds present in meteorites and comets to the origin of life on Earth.
She has a 5-year undergraduate degree (Licenciatura) in Chemistry from Instituto Superior Técnico (Portugal, 2002) and a PhD in Astrobiology from Leiden University (The Netherlands, 2007). She was an Invited Scientist at NASA Goddard, and an Invited Professor at the University of Nice-Sophia Antipolis (France). In 2009 she was awarded a Royal Society Research Fellowship worth 1 Million British Pounds.
She is a Co-Investigator of two space missions (OREOcube and EXOcube), which will be installed in the International Space Station. Zita Martins is a Member of two Topical Teams of the European Space Agency (ESA), a Member of the Phobos Sample Return Science Study Team of ESA, a Member of the UK Space Agency's Space Environments Working Group, and a Committee member of the Astrobiology Society of Britain (ASB).
Zita Martins has an active involvement with public outreach activities, including several international media interviews and public talks at museums and science festivals (more info at http://www3.imperial.ac.uk/people/z.martins and Twitter https://twitter.com/ZitaMartins). She had the honour of having her portrait sketched (special commission) for the Royal Society exhibit about successful women in science. She was also selected by the BBC as an Expert Women Scientist.
In 2015 Zita Martins was appointed Oficial da Ordem Militar de Sant'Iago da Espada (OSE) by the President of Portugal for exceptional and outstanding merits in science.

Origin and detection of life in our solar system

The question of how life originated on Earth is yet to be answered by scientists. It is currently accepted that a continuous supply of organic molecules was as a requirement for the origin of life. These organic molecules may have been synthesized on the early Earth (by atmospheric and/or geochemical sources, e.g. in deep sea vents), and/or delivered by exogenous sources (e.g. comets and meteorites). Indeed the Earth was heavily bombarded by comets, asteroids and meteorites 4.6 to 3.8 billion years ago. Life may have also originated in other parts of our solar system, including Mars and some of the icy moons of Jupiter and Saturn (e.g. Europa and Enceladus). In this talk we will discuss how exogenous sources may have contributed to the origin of life in our planet, and how to detect potential signatures of extra-terrestrial life in our solar system.

ORAL PRESENTATIONS

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O1 - Formulation of pea protein for increased satiety and improved foaming properties

Cátia S. Carmo[1], Ana N. Nunes[1], Inês Silva[1], Catarina Maia[1], Joana Poejo[1], Susana Ferreira-Dias[2], Isabel Nogueira[3], Rosário Bronze[1,4] and Catarina M. M. Duarte[1]

[1] Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal and Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
[2] Instituto Superior de Agronomia, LEAF, Universidade de Lisboa, Portugal
[3] Instituto Superior Técnico, Instituto de Ciências e Engenharia de Materiais e Superfícies, Universidade de Lisboa, Portugal
[4] Faculdade de Farmácia da Universidade de Lisboa, Avenida das Forças Armadas, 1649-019 Lisboa, Portugal

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Pea protein (PP) has been associated with promoting satiety effect. One of the issues associated with the incorporation of pea protein in food products is the product homogeneity due to its solubility and dispersibility issues. Within this context, one goal of this study was to exploit the use of Supercritical Fluid Technology to develop Solid Lipid Pea Particle (SLPP) aiming at improving dispersibility in fat-based products. PP was encapsulated by PGSS® (Particles from Gas Saturated Solutions) technique into glyceryl dipalmitostearate (E471) and olive oil. Different process conditions, namely pressure (7.3-20.7MPa), temperature (51-75˚C) and equilibrium time (3-37min) were tested in order to optimize the encapsulation of pea protein via Response Surface Methodology (RSM), following a Central Composite Rotatable Design (CCRD). Results showed that pressure and the interaction between pressure and temperature had a significant impact ($p<0.05$) on the protein load and thus on the encapsulation efficiency. The highest encapsulation efficiency (96%) was achieved at 14 MPa, 51˚ C and 20 min. At these conditions, SLPP presented 0.15mg of protein/mg of particles and 84% of lipase inhibitory activity. When compared with the PP (non-encapsulated), liposoluble pea protein particles contributed to a better product homogenization. The food industry can also take advantage of the ability of pea protein for foam stabilization in aqueous food products. Therefore, PP was treated with High-Pressure Supercritical CO_2 Treatment (HPT-scCO_2) that has led to improved foaming properties when compared with the non-treated PP.

Keywords: Pea protein; Particles from Gas-Saturated Solutions (PGSS®; satiety; High-Pressure Supercritical CO_2; foam stability

O2 - Biocatalytic interconversion of H_2 and formate

Cláudia Mourato[1], Mónica Martins[1] and Inês A.C. Pereira[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA),Universidade Nova de Lisboa, Oeiras, Portugal.

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The great dependence on fossil fuels and consequent emission of CO_2 is a major cause of environmental pollution and global climate change. Therefore, there is an urgent demand to find alternative energy sources and new mechanisms to reduce the levels of CO_2 in the atmosphere. Hydrogen is one of the most attractive candidates as an alternative energy carrier since it has a high energy density and is carbon neutral. However, it is important to find a clean and inexpensive process to produce and store it. Formate has been viewed as an environmentally friendly H_2 storage material since it is liquid at room temperature, non-toxic and non-flammable and can thus be handled and transported easily. One efficient system for the production of H_2 from formate is through the use of microorganisms as biological catalysts. In addition, recent studies have also proven the potential of biocatalysts to produce formate from the hydrogenation of CO_2, which is a promising biotechnological application for the sequestration of CO_2 and a means of H_2 storage.[1] Sulfate-reducing bacteria (SRB) are anaerobic bacteria with a high content of formate-dehydrogenases (FDHs) and hydrogenases (Hases), the enzymes responsible for the reversible reactions of H_2 and formate production,[2,3] making them good candidates for production of H_2 and formate. In this work, we will report studies on the use of SRB for formate-driven H_2 production,[3,4] as well as, the conversion of CO_2 and H_2 to formate.[5]

References:
1. Schuchmann K & Müller V. (2013) Science 342, 1382-5
2. Pereira IAC *et al.* (2011) Front Microbiol 2, 69
3. Mourato C *et al*. (2016) Appl Microbiol Biotechnol 100, 8135-46
4. Martins M *et al*. (2015) Environ Sci Technol 49, 14655-62
5. Mourato C *et al*., in preparation

Keywords: H_2 production, Formate production, Sulfate-reducing bacteria, Biocatalysts, Formate-based H_2 storage system

O3 - Assessing antinutritional compounds and protein quality of different Portuguese common bean (*Phaseolus vulgaris L.*) varieties

Elsa Mecha[1], Maria Eduardo Figueira[2], Maria Carlota Vaz Patto[1] and Maria do Rosário Bronze[1,2,3]

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[2] Faculdade de Farmácia, Universidade de Lisboa, Av. Das Forças Armadas, 1649 – 019 Lisboa, Portugal
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Common beans (*Phaseolus vulgaris L.*) are grain legumes widely distributed in the world. As a consequence of genetic domestication, during centuries, small-scale farmers selected and preserved different varieties adapted to distinct environmental conditions. Until now there is a scarcity of information regarding the characterization of nutritional and anti-nutritional compounds in Portuguese varieties. Although legumes represent a crop rich in vegetable protein, essential micronutrients (e.g folate, zinc and iron) and antioxidant compounds (e.g phenolic compounds) for human health, the level of consumption had fallen in several world regions. Under the framework of an international project, SOLIBAM – Strategies for Organic and Low-Input Integrated Breeding and Management and a Portuguese Project – BEGEQA – Exploiting Bean Genetics for Food Quality and Attractiveness Innovation, 108 different Portuguese common bean varieties were multiplied and analyzed following different analytical methodologies. In a first approach, the phenolic composition and in vitro antioxidant activity of 31 different common bean samples were assessed by spectrophotometry considering different common bean fractions (whole flour, coats, cotyledons and corresponding soaking water). Quantification of some phenolic compounds (phenolic acids and flavonoids) was performed by UPLC-Q-TOF-MS in a larger set of samples. Phenolic compounds' metabolites formed after consumption of cooked common beans were quantified in plasma and urine to elucidate for the first time inter-individual differences in bioavailability of phenolic compounds metabolites. A high variability in aminoacid content was detected in this common bean collection by HPLC-FLuorescence detection. Heat treatment contributed to increase significantly *in vitro* protein digestibility. Other parameters such as phaseolin type and enzymatic inhibitors content will be considered in future to provide valuable information for breeding program.

Keywords: *Phaseolus vulgaris L.*; Spectrophotometry; Chromatography; Bioavailability; Quality

O4 - Cell-based biosensors for detection and quantification of label-free virus for research and diagnostics

Miguel Ricardo Guerreiro[1,2], Ana Filipa Rodrigues[1,2], Paula Marques Alves[1,2] and Ana Sofia Coroadinha[1,2]

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Viruses are pathogenic agents that can cause a wide span of serious diseases, driving extensive efforts towards the development of new vaccines and antiviral agents. Both clinical and research applications for virus and viral vectors require reliable detection and quantification assays. However, current methods fail to provide it, meeting at least one of the following pitfalls: extremely time-consuming, lack high-throughput potential, or require the use of labels on the virus which may have a negative impact on virus biology and are unacceptable in a human therapeutic or prophylactic context.

Herein, we report the development of genetically encoded fluorescent biosensors for detection and quantification of label-free viruses: VISENSORS. These conditionally-fluorescent biosensors are triggered by viral enzyme activity – thus a label-free system – and can be stably expressed into suited mammalian cell lines. Different designs of biosensors for different viruses and viral vectors are currently being optimized by means of molecular biology.

VISENSORS' potential was confirmed by establishing HEK 293 sensor cell lines for the detection of recombinant Human Adenoviruses and HIV-based lentiviruses. Analyses by flow cytometry showed a signal to noise up to 4 – in line with previously reported mammalian cell-based conditionally-fluorescent biosensors – leaving room for further improvements. Validation against current standard detection and quantification assays and high-throughput antiviral screening potential will follow.

VISENSORS have the potential to deliver fast and accurate detection and quantification of clinically relevant label-free virus and viral vectors, currently missing in the vaccines and antiviral development fields. VISENSORS will also allow the establishment of new cellular platforms not only for diagnostic and clinical applications but also for basic virology research.

Keywords: Animal Cells, Fluorescent Proteins, Biosensors, Label-free virus, Flow cytometry

O5 - Tailor-made plant cell cultures for optimized production of recombinant proteins

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Molecular Farming - the use of plant based systems for production of recombinant proteins - is an emerging field with a high potential impact in the biopharmaceutical industry. Plant production platforms arose as promising alternatives to traditional expression systems, since they offer significant advantages, in terms of safety and cost. However, a major challenge remains so that plants become truly competitive, which is to improve the low production yields that are generally obtained.

We have developed strategies for the improvement of human recombinant protein production in *Medicago* cell suspension cultures. Our aim is to enhance the transcription rate of transgenes by manipulating the cell's epigenetic profile. At the same time, we aim to reduce the proteolytic activity in this system, which affects final recombinant protein yields.

To enhance the transcription rate, we tested the addition of commercial compounds to our cell cultures. These compounds are known to inhibit histone deacetylases, promoting the hyperacetylation of DNA and, therefore, improving transcription by opening the chromatin. We assessed the compound effect on transgene expression by digital PCR and the effect on recombinant protein production by western blotting. We also analyzed the level of histone acetylation.

To reduce proteolytic activity, we studied the proteolytic profile of proteases present in our system by analyzing the degradation pattern of target proteins. With the addition of specific protease inhibitors, we could detect which protease classes are present. We used ABPP probes to identify specific protease, within the protease class identified that is affecting target proteins. In parallel, we performed mass spectrometry analysis of the plant suspension media. Finally, we proceeded to co-express protease inhibitors in the *Medicago* system, reducing proteolytic activity.

Together, these strategies aim to improve the recombinant protein production yields in *Medicago* cell cultures.

Keywords: *Medicago truncatula*, Plant Cell Cultures, Recombinant Protein, Proteases, Epigenetics

O6 - Metabolic fingerprint of mAb-producing GS-CHO cells using labelled tracers

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Chinese hamster ovary (CHO) cells are preferred mammalian hosts for industrial production of therapeutic glycoproteins. With the advent of systems biology tools it became possible to generate a more comprehensive knowledge of cellular physiology for posterior cell engineering. In order to obtain an in depth view of cell metabolism, namely the relative activities of parallel pathways and substrate contributions to intracellular metabolites, we performed tracer studies using labelled tracers of the three most consumed nutrients ([1,2-^{13}C]glucose, [U-^{13}C/^{15}N]asparagine and [U-^{13}C/^{15}N]serine) followed by GC-MS and LC-MS. The patterns produced from the [1,2-^{13}C]glucose tracer revealed a high diversion of glucose to the pentose phosphate pathway when compared to that previously published for non-producing CHO cells during the same growth phase, suggesting this route as an important source of NADPH for recombinant protein synthesis. Another potential source of NADPH is the flux through malic enzyme, the high activity of which was suggested by the labelling pattern of pyruvate after incubation with [U-^{13}C/^{15}N]asparagine. Noteworthy, asparagine was essential for replenishing TCA cycle intermediates as well as for glutamine synthesis in GS-CHO cells. This information could be used to generate hypotheses on how different metabolic profiles confer the respective and also different productivities.

Acknowledgements: Support provided by FCT (SFRH/BD/81553/2011 and PTDC/BBB-BSS/0518/2012).

Keywords: CHO cells, MS, parallel experiments, ^{13}C-isotopic tracers, metabolism

O7 - Identification and functional characterization of transcription factors regulating *ZmPEPC1* in maize

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C4 photosynthesis evolved from the C3 metabolism and C4 plants show an improved photosynthetic efficiency. The advantage of C4 plants results from a specific leaf anatomy and compartmentalization of the photosynthetic reactions between two leaf cell types: mesophyll and bundle sheath. The maize C4 Phospoenolpyruvate carboxylase (*ZmPEPC1*) is a key C4 photosynthetic gene and is specifically expressed in mesophyll cells. Interestingly, previous studies have shown that the promoter of *ZmPEPC1* fused to GUS reporter gene directs cell specific and light regulated expression in C3 plant, rice.[1] This observation indicates that rice has the transcriptional machinery necessary to regulate *ZmPEPC1* gene expression. Here, we report the identification and functional characterization of novel transcription factors regulating *ZmPEPC1* gene expression. Using a Yeast One Hybrid approach, we have identified one rice (OsbHLH) and five maize (ZmbHLHs, ZmOrphan, ZmHB, ZmCPP) transcription factors binding to the maize *PEPC1* promoter. Importantly, we have shown that the maize orthologs of identified rice OsbHLH also interact with *ZmPEPC1* promoter, highlighting the importance of this regulation in *ZmPEPC1* gene expression. The cis-regulatory element within *ZmPEPC1* promoter to which the OsbHLH maize ortholog binds has been identified and validated by EMSA. Using a Yeast Two-Hybrid and BiFC assays we have shown that novel maize TFs form heterodimers. A trans-activation assay in maize protoplasts has been performed in order to understand how the TF – TF interactions regulate *ZmPEPC1* gene expression. In addition, a transgenic approach is being used to determine the biological function of the most interesting TFs.

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Keywords: C4 photosynthesis, *Zea mays*, *ZmPEPC1*, Transcriptional regulation, Cell-specific gene expression

O8 – Unraveling the mechanisms of extracellular respiration of thermophilic Gram-positive bacteria

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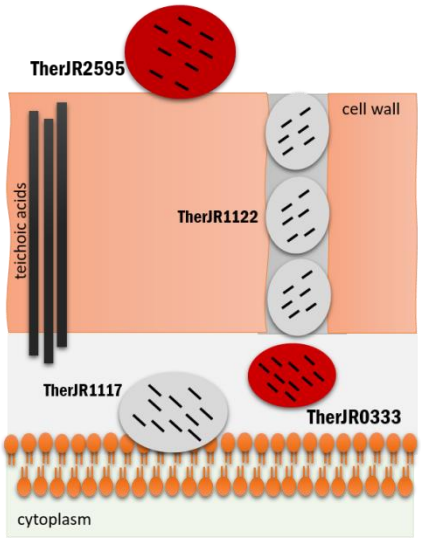
Microbial electrochemical technologies are emerging as environmentally friendly biotechnological processes. Recently, an electrogenic thermophilic Gram-positive bacterium was isolated from a microbial fuel cell (MFC) operating at 55ºC. This bacterium contains several multiheme *c*-type cytochromes that are the key players of extracellular electron transfer pathway, which drives electricity production in MFCs. In order to understand the molecular basis by which Gram-positive bacteria perform this type of respiration, these proteins need to be characterized in detail. Toward this objective we developed a strategy to heterologously express and purify the multiheme cytochromes from the Gram-positive bacterium *Thermincola potens* JR. This strategy will open the possibility for further studies involving heterologous expression of Gram-positive multiheme cytochromes. Up to date, two cytochromes were successfully expressed and purified. These are the decaheme periplasmic protein TherJR_0333 and the nonaheme outer-surface protein TherJR_2595 that is the presumed terminal reductase. Spectroscopic techniques were used to characterize structurally and functionally these cytochromes, with the aim of understanding the extracellular electron transfer pathway used by these organism. This knowledge will be of significant importance to guide the rational improvement of Microbial Electrochemical devices for energy harvesting and wastewater treatment using thermophilic organisms.



Hipothetical extracelullar electron transfer pathway of *T.Potens JR* (adapted from Carlson et. al PNAS 2011)

Keywords: Heterologous Expression, *Thermincola*, Multiheme *c*-type Cytochromes, NMR, Extracellular Electron Transfer

O9 - Flavodiirion proteins: a structural approach to understand the substrate selectivity

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Flavodiiron proteins (FDPs) are part of the biological response machinery for oxygen and/or nitric oxide detoxification, including higher plants. The minimal structural unit of FDPs is composed of a metallo-β-lactamase-like and a C-terminal flavodoxin-like domain. The first domain contains a diiron catalytic site where reduction of O_2 (to H_2O) and/or NO (to N_2O) occurs, and the second domain contains an FMN.[1, 2]

The *E. coli* FDP contains an additional rubredoxin domain at the C-terminus, being named flavorubredoxin (EcFlRd).[3] EcFlRd is a nitric oxide reductase (NOR) that protects *E. coli* against NO.[4] It is so far the only known FDP exclusively reactive toward NO and its structure was recently determined.[5] A comparison with the crystal structure from the NO-reducing EcFlRd and a structural model of the O_2-selective FDP from *Entamoeba histolytica* (EhFDP) was performed aiming to unveil the structural determinants that define FDPs substrates selectivity.

Differences were observed at two positions within the diiron second coordination sphere and kinetic properties of single and double mutants in EhFDP showed that its reactivity with O_2 was affected, whilst the NO consumption rate increased.[6]

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

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

O10 - Unravelling the molecular mechanisms that orchestrate the electron transfer from inside *Shewanella oneidensis* cells to terminal reductase cytochromes

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Among the portfolio of proposals for sustainable industrial processes, Microbial Electrochemical Technologies (METs) are emerging as a versatile option with multiple potential applications, such as bioremediation of metal contamination, wastewater treatment, and electricity production in microbial fuel cells. *Shewanella oneidensis* MR-1 (SOMR-1) is a sedimentary Gram-negative bacterium with a highly versatile metabolism, uniquely suited for the operation of METs, due to its ability to link its bioenergetic metabolism through the periplasm to reduce extracellular electron acceptors. The present work is focused on understanding the electron transfer pathways orchestrated by this bacterium from inside the cells to the final extracellular electron acceptor with the goal of optimizing the electron transfer in METs.

To understand if the electron transfer pathways across the periplasm are specific for each terminal electron acceptor, or if they are common for the different respiratory processes, NMR and UV-visible spectroscopy were used. STC, a tetraheme cytochrome and one of the most abundant proteins in the periplasm of SOMR-1 was shown to be a promiscuous mobile redox protein responsible for the periplasmic electron transfer to iron-, nitrite- and DMSO-reductases.

At the cell surface, OmcA is the most abundant decaheme cytochrome located on the outer surface of SOMR-1 cells and the major responsible for extracellular electron transfer to terminal electron acceptors and electron shuttles. Characterization of the interactions between OmcA and its physiological partners (iron oxides and/or mobile electron shuttles) was performed using NMR and stopped-flow experiments. Using site directed mutagenesis, the hemes directly involved in electron transfer from OmcA to the final electron acceptors, were identified.

Overall, these results provide guidance for the design of mutated SOMR-1 strains optimized for different operational modes of microbial electrochemical devices.

Keywords: *Shewanella oneidensis* MR-1 (Gram negative bacteria), NMR, Electron transfer pathways, protein-protein and protein ligand interactions, Cytochromes

O11 - Drought and subsequent recovery behaviour of the biodiesel plant *Jatropha curcas*: Integrating transcriptomics and physiology

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Jatropha curcas, a multipurpose plant attracting attention due to its high oil content and quality for biofuel, is recognized as a drought-tolerant species. However, this drought tolerance is still poorly characterized. This study aims to contribute to uncover the molecular background of this tolerance, using a combined approach of transcriptional profiling and morphophysiological characterization during a period of water-withholding (49 d) followed by rewatering (7 d). Morphophysiological measurements showed that *J. curcas* plants present different adaptation strategies to withstand moderate and severe drought. RNA sequencing was performed for samples collected under moderate and severe stress followed by rewatering, for both roots and leaves. *J. curcas* transcriptomic analysis revealed shoot- and root-specific adaptations across all investigated conditions, except under severe stress, when the dramatic transcriptomic reorganization at the root and shoot level surpassed organ specificity. These changes in gene expression were shown by the down-regulation of genes involved in growth and water uptake, and up-regulation of genes related to osmotic adjustments and cellular homeostasis. However, organ-specific gene variations were also detected, such as up-regulation of abscisic acid synthesis in roots under moderate stress and of chlorophyll metabolism in leaves under severe stress. Functional validation further corroborated the differential expression of genes coding for enzymes involved in chlorophyll metabolism, and correlated with the metabolite analyses of this pathway.

Acknowledgments: Funding by FCT through Project PTDC/AGRGPL/101435/2008, and the fellowship grants SFRH/BD/89781/2012 (HS), SFRH/BPD/34429/2006 (JMC), SFRH/BPD/34943/2007 (TL) and the Research unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013).

Keywords: *Jatropha curcas*, RNA-Seq, drought–recovery, gene profiling, leaf and root.

O12 - Unveiling the key players of the rice SUMOylation machinery in drought response

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Rice is one of the most important world food crops, but extremely sensitive to abiotic stress conditions, including drought. Thus, it is crucial to understand the molecular mechanisms underlying stress tolerance. SUMOylation is a post-translational modification associated with stress response, regulating numerous cellular processes. The goal was to establish which SUMOylation machinery genes are key in rice drought response.

Our results show that SUMOylation should be considered as an exceptionally regulated pathway that modulates cellular response in a tissue-dependent manner. Within the SUMOylation machinery, gene promoter regions analysis showed the existence of drought-responsive cis-elements. These elements are highly represented in the promoter of the catalytic subunit of the SUMO activating enzyme. This evidence, together with low basal mRNA expression levels and strong upregulation during drought and cold, suggested OsSAE2.2 as a possible regulatory step of the SUMOylation process in response to stress. To further support this, we found that *OsSAE2.2* overexpression can significantly increase plant yield under stress. Moreover, we show that within the E2 conjugase rice family, *OsSCE1b* is the main constitutive E2, while *OsSCE1a/c* appear to have specific functions related to stress response. For functional validation several SUMOylation machinery genes were overexpressed, in which *OsSIZ1* was the most promising element, leading to enhancement of several important biomass- and seed-related parameters under drought. Overall, we proved that the SUMOylation machinery could be used to improve rice fitness under drought.

Keywords: Rice, qPCR/Western blot, drought response, SUMOylation, alternative splicing

O13 - Study of seasonal weight loss tolerance in small ruminants – an NMR-Metabolomics approach

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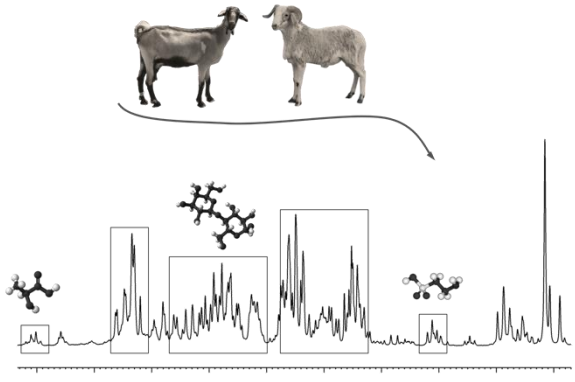
Small ruminants are very important in the tropics and the Mediterranean areas. In these regions, production is strongly affected by pasture scarcity during the dry season, which leads to Seasonal Weight Loss (SWL), one of the most important problems. However, some breeds present higher tolerance to SWL. Understanding the physiological mechanisms by which breeds are able to cope with SWL is of utmost importance in animal selection. We studied two dairy goat breeds (*Majorera* and *Palmera*) and three breeds of meat-producing sheep (*Merino*, *Dorper* and *Damara*), with different levels of tolerance to SWL. The aim of this study was to characterize the metabolome of the mammary gland and milk of the goats, and the metabolome of muscle and liver in sheep. We used Nuclear Magnetic Resonance to assess metabolome profiles and Gas Chromatography to determine the fatty acid composition. Goats and sheep were studied in two different work tasks. In both species, animals were divided in control and restricted-fed groups for the different breeds.

Concerning dairy goats, significant differences were observed in mammary gland and milk between control and restricted groups, albeit with no differences between breeds. Fatty acid analysis revealed breed effect in the response to feed-restriction. In general, *Majorera* breed is more adapted to the effects of SWL. Milk presented significant variations due to feed-restriction and seems to be more susceptible to these variations than the mammary gland.

Regarding the sheep breeds, *Dorper* showed less changes in both tissues, whereas *Merino* presented more differences between control and restricted-fed groups, mainly related with fat and protein mobilization. *Damara* presented an intermediate response, with differences in amino acids composition in muscle and in energy-related pathways in liver. *Dorper* and *Damara* breeds are more tolerant to SWL conditions and thus more suitable for selection in harsh environmental conditions.



Keywords: Goat, Sheep, NMR, Seasonal Weight Loss, Metabolomics

O14 - Wolverine and SLR1, the only DELLA protein in rice: Separated At Birth

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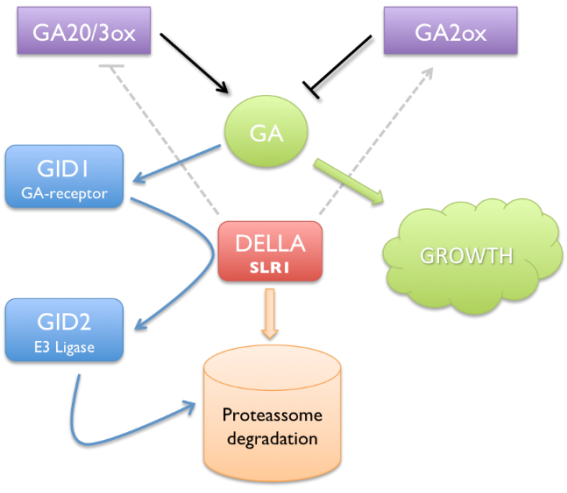
DELLA proteins have been classically described as repressors of gibberellin response. Their regulation mainly occurs at the post-translational level, with several post-translational modifications (PTMs) enhancing their repression activity. In *Arabidopsis* there are five DELLA proteins, while in rice, like in most cereals, only one exists, SLR1.

With the knowledge that DELLA proteins are connected to salt tolerance in *Arabidopsis* and given that rice is the most sensitive cereal to high-salinity conditions, our aim is to characterize SLR1 and its role in response to salt stress. We observed that rice shows both differential tissue-specific and salinity-induced SLR1 accumulation. Currently, we are engaged in establishing a correlation between higher SLR1 protein levels and enhanced stress tolerance in commercial rice cultivars.

A new layer of post-translational regulation of DELLAs in Arabidopsis was recently added, with increased protein stability conferred by SUMOylation. We determined that SLR1 is SUMOylated *in vitro*. We were able to confirm that in rice this PTM occurs in a different residue from the one reported in *Arabidopsis*. We are presently working to unveil the role of SUMOylation in rice and how SUMOylation regulates specific SLR1 protein stability.

These are the initial steps in unveiling SLR1 function in abiotic stress and the fine-tuning of its regulation by PTMs. How it differs from the current *Arabidopsis* DELLA model is crucial to ensure the application of this knowledge in cereals, as it was recently reported that SLR1 integration of several hormonal pathways in response to biotic stresses is distinct from the orthologue mechanism in *Arabidopsis*.



Thus, we expect our contribution in the understanding of DELLA abiotic stress tolerance mechanisms in rice to be used in the design of better adapted crops, able to grow in increasingly challenging conditions.

Keywords: Rice, Proteomics, Biochemistry

O15 - Transcriptional and post-transcriptional mechanisms underlying cadmium detoxification in yeast

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Cadmium is a well-known mutagenic metal, which can enter the cell via non-specific metal transporters, causing several cellular damages and eventually leading to death. In the yeast *Saccharomyces cerevisiae*, the transcription factor Yap1 plays a key role in the regulation of several genes involved in metal stress response. We have previously shown that Yap1 represses the expression of *FET4*, a gene that codes for a low affinity iron transporter able to transport metals other than iron.[1] Here, we have studied the relevance of this repression in cell tolerance to cadmium. Our results indicate that genomic deletion of Yap1 increases *FET4* transcripts and protein levels. In addition, cadmium toxicity exhibited by this strain is completely reversed by co-deletion of *FET4* gene. This data correlates well with the increased intracellular levels of cadmium observed in the mutant *yap1*. Rox1, a well-known aerobic repressor of hypoxic genes, conveys the Yap1-mediated repression of *FET4*, as we found its expression to be directly dependent on Yap1. We have further shown that, in a scenario where the activity of Yap1 or Rox1 is compromised, cells activate post-transcriptional mechanisms, involving the exoribonuclease Xrn1, to compensate the de-repression of *FET4*.[2]

Our data reveals therefore a novel mechanism of protection against cadmium toxicity mediated by Yap1 that relies on the aerobic repression of *FET4* and results in the decrease of cadmium uptake.

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Keywords: Yeast, metal detoxification, stress response, gene regulation

O16 - Novel synthesis of Oseltamivir and Tamiphosphor and other synthetic methodologies

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Oseltamivir is a drug used for the treatment of influenza virus infections. It is commercialized by Roche under the name Tamiflu. New synthetic routes for this drug, as well as its analogues, are still a great challenge for chemists.[1] Tamiphosphor is an example of an interesting analogue. It has shown to be more potent than Oseltamivir and effective against resistant strains.[2] In this work a new synthetic route for both Oseltamivir and Tamiphosphor was developed. The early formation of aziridine moiety is a key step, not used in previous syntheses. Also the resolution of intermediate 1 constitutes an efficient asymmetric approach, with almost quantitative yield and very high enantiomeric excess. Its conversion into oseltamivir was achieved in 10 steps and 22% yield and into Tamiflu diethyl phosphonate in 12 steps and 19% yield.

The progress of organic synthesis is closely dependent on the development of new improved reactions: more efficient, selective, economical or greener. In the present work, two new reactions with a great synthetic potential are also described. One is a method for the basic α-chlorination of ketones and other functional groups, in which methyl chlorosulfate is described as a chlorine source for the first time. It is a very clean reaction with great yields. The second is a method for the transformation of 2-oxo S-carbonyl or thiocarbonyl into 2-oxo (thio)carbonyl compounds by a base promoted sulfur abstraction rearrangement. It is a very clean reaction and products are obtained in good yield in just 30 minutes.

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Keywords: organic chemistry, synthesis, oseltamivir, tamiphosphor

O17 - Crossing the blood-brain barrier: effects of human bioavailable (poly)phenol metabolites in neuronal cells

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In the past decades, studies have revealed that diets rich in fruits and vegetables promote health benefits, preventing against age-related disorders. Such pathologies are still cure-less and berries emerge amongst the most promising fruits, rich in (poly)phenols, increasingly regarded as effective protectors. Evidences indicate that some (poly)phenol metabolites can enter in the central nervous system in very small amounts but little is known about their true effects inside the brain.

Here we studied the putative transport of bioavailable (poly)phenol metabolites across the blood-brain barrier (BBB), their biotransformation and ultimate beneficial effects that these metabolites may hold in neuronal cells. (Poly)phenol metabolites were identified and quantified circulating in a human intervention study with berries. Metabolites were chemically synthesized and used in cell assays at physiological concentrations. We profited from a BBB cell model to assess transport and further metabolization. We observed, by LC Orbitrap-MS, that these (poly)phenol metabolites are differently transported and new end-route metabolites were identified. The studied (poly)phenol metabolites have also shown protective potential in different neuronal cell systems, towards an oxidative insult and an inflammatory stimulus. This work highlights the pertinence of study (poly)phenol metabolites arising from our diet since we observed their BBB permeability and detected new end-route metabolites. Moreover, their neuroprotective and anti-inflammatory activity open new avenues for further mechanistic studies that should be extended for the new end-route metabolites. Overall this study will impact our understanding how diet could contribute for a healthier brain.

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Keywords: (Poly)phenols, blood-brain barrier, neuroprotection

O18 - Single N-terminal phosphorylation modulates mutant huntingtin aggregation and toxicity

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Huntington's disease (HD) is an unstoppable and fatal neurodegenerative disorder caused by a polyglutamine expansion in the huntingtin (HTT) protein. Double serine 13/serine 16 phosphorylation in the N-terminus of HTT (NT17) prevents its aggregation and toxicity *in vivo*, but this double phosphorylation is neither frequent nor constitutive. Here, we analyzed the relative contribution of each phosphorylatable residue (Thr3, Ser13 and Ser16) in the NT17 region towards HTT exon 1 oligomerization, aggregation and toxicity *in vitro* and *in vivo*, in *Drosophila*. Single phosphomimic mutations completely abolished HTT aggregation in cultured human cells. In *Drosophila*, HTT aggregation patterns depend on age or cellular context. Strikingly, and in line with these observations, we found that protein phosphatase 1 inhibitors prevented HTT aggregation and toxicity *in vitro* and *in vivo*. In total, our findings suggest that modulation of HTT phosphorylation at specific sites might constitute an efficient and direct molecular target for therapeutic interventions against HD.

Keywords: Huntington's Disease, phosphorylation, bimolecular fluorescence complementation, *Drosophila melanogaster*, neurodegeneration

O19 - Colorimetric detection of pyrophosphate in water with dinuclear zinc(II) complexes of *m*-xylyl-bis-cylen

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The detection of anions by synthetic receptors in water is an important task in the analysis of biological systems, medical diagnostics, and environmental monitoring.[1,2] Achieving this goal for phosphorylated anions, that are crucial for energy storage and transcriptional activity in biological systems, has become of great relevance.[3] The sensing of pyrophosphate (PPi), has been specially studied due to its importance in several biological processes and diseases.[4]

In this work, a new member of the *m*-xylyl-bis-cyclen family, **tmbce** with a 1,3,5-triethylbenzene spacer was synthesized, and the parent **mbce** was revisited in order to perform a comparative study of both compounds and their complexes. The dinuclear copper(II) and zinc(II) complexes of the two ligands were studied in parallel and their selectivity towards phosphorylated substrates evaluated. Two new X-ray crystal structures for both dinuclear copper(II) complexes were obtained. Furthermore, an Indicator Displacement Assay (IDA) revealed that the $[Zn_2\mathbf{mbce}]^{4+}$ receptor is selective for $HPPi^{3-}$ over HPO_4^{2-}, PhP^{2-}, AMP^{2-}, ADP^{3-} and ATP^{4-} at physiological conditions with changes visible to the naked eye (Fig. 1).

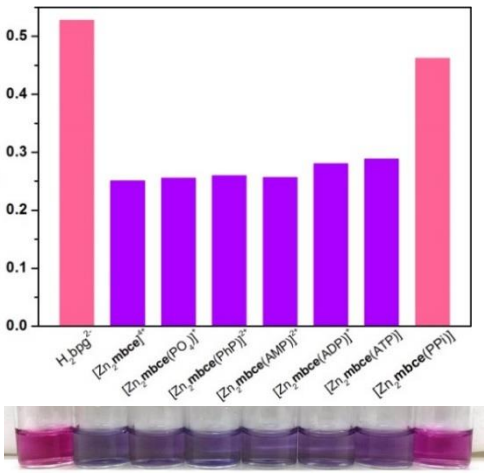

Figure 1. Sensing of $HPPi^{3-}$ in a buffered aqueous solution ([MOPS] = 20 mM, pH 7.4).

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Keywords: macrocycles, bis-cyclen, molecular recognition, pyrophosphate, zinc complexes

O20 - Staying in shape: *Staphylococcus aureus* FtsZ-dependent morphogenesis of elongated cells

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Mechanistic understanding of determination and maintenance of the simplest bacterial cell shape, a sphere, remains elusive compared with more complex shapes. Cocci seem to lack a dedicated elongation machinery, and spherical shape has been considered an evolutionary dead-end morphology, as a transition from spherical to rod-like shape has never been observed in bacteria. We have now shown that a *Staphylococcus aureus* mutant (M5) expressing the *ftsZ*^{G193D} allele exhibits elongated cells. Molecular dynamics simulations indicate that FtsZ^{G193D} filaments are more twisted than wild-type. *In vivo*, M5 cell wall deposition is initiated asymmetrically, only on one side of the cell and progresses into a helical pattern, rather than into a constricting ring as in wild-type cells. This helical pattern of wall insertion leads to elongation, like in rod-shaped cells. Thus, structural flexibility of FtsZ filaments can result in an FtsZ-dependent mechanism for generating elongated cells from cocci.

Keywords: Bacteria; super resolution microscopy; morphogenesis; cell division; FtsZ

O21 - Genomic approaches to understand the responses of *Castanea* spp. to infection by *Phytophthora cinnamomi* Rands

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Chestnut orchards and forests are declining in Europe due to ink disease, caused by the destructive and widespread oomycete *Phytophthora cinnamomi* (Pc). Pc infection occurs in roots causing rot and dieback in susceptible species. However, susceptible level varies among chestnut species, being the Asian species the most resistant to Pc. A Portuguese chestnut breeding program, based on controlled crosses made between the resistant Japanese chestnut and the susceptible European chestnut was established 10 years ago. Mapping and transcriptomic approaches have been implemented aiming to unveil the different mechanisms of disease response across chestnuts. So far, 155 progenies were obtained, genotyped and phenotyped in order to map genomic regions controlling Pc resistance (Quantitative Trait Loci-QTLs). Using microsatellite and single nucleotide polymorphism (SNP) as molecular markers, genotypic data was collected and used for the construction of the first European x Japanese chestnut genetic map. Phenotyping was performed by evaluating diverse disease metrics through Pc inoculation of roots and/or of excised shots.[1] The association between genotype and phenotype enabled the mapping of QTLs for Pc resistance.

Additionally, candidate genes for Pc resistance were selected from the root transcriptome of European and Japanese chestnut inoculated and non-inoculated with Pc.[2] New microsatellite markers were developed from those candidate genes and were used for genetic map saturation.[3] Furthermore, expression levels of 8 genes were quantified by digital PCR, using different species and hybrid genotypes.[4] The knowledge acquired in this project is a major breakthrough for the understanding of chestnut-Pc interactions and may contribute for the development of strategies to control ink disease.

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Keywords: Plants, Molecular Genetics, Pathogen resistance, *Castanea* spp., *Phytophthora cinnamomi*

O22 - Treatment of aqueous effluents contaminated with active pharmaceutical ingredient (APIs)

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Recently, a large increase worldwide consumption of pharmaceutical drugs occurred as a result of the need to significant improves human living conditions. Over the past few years, development of advanced analytical tools and investigation on wastewater samples confirmed the presence of residual amounts of active pharmaceutical ingredients (APIs) in wastewater treatment plants (WWTPs), sewage treatment plants (STPs), groundwater and drinking water.

Once variable amounts of the taken doses of APIs are metabolized by mammals, the remaining is excreted in a metabolized or unchanged form. Even at low detection concentrations (ng/L – µg/L), the contact with APIs can lead to the intoxication of living organisms. Thus, numerous studies have been showing that the wide variety of pharmaceuticals present in wastewater effluents is a matter of great concern for the public and animal health.

In this context, this thesis addresses the development and study of two processes already well-documented in WWTPS, namely liquid-liquid extraction (LLE) and solid-phase extraction (SPE), making use of a novel class of compounds – ionic liquids (ILs). Ionic liquids present excellent solvating qualities, which can be additionally tailored to guarantee the success of specific extractions. Once non-steroidal anti-inflammatory drugs (NSAIDs) and fluoroquinolones (FQs) are the main APIs detected in aquatic environment, the principal goal of the current work is the use of aqueous biphasic systems (ABS) composed of ionic liquids (ILs) (LLE technique) and supported ionic liquid phase (SILP) (SPE technique) for a complete pharmaceutical drugs removal from aquatic environment.

Keywords: Active pharmaceutical ingredient, wastewater treatment plants, liquid-liquid extraction, solid-phase extraction, ionic liquids

O23 - Insect cells platforms for fast production of pseudo-typed VLPS for drug and vaccine development

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Expression systems capable of delivering high concentrations of membrane proteins in their native structure are essential in the vaccine field as well as in drug discovery. In this work, we took advantage of insect cell expression and site-specific gene integration based on flipase-mediated cassette exchange (FMCE) technology to generate cell platforms for efficient production of membrane proteins on the surface of a protein scaffold, namely enveloped virus-like particles (VLPs). The expression of membrane proteins concomitantly with capsid proteins of enveloped viruses (e.g. HIV Gag or influenza M1) will enable their capturing in lipid rafts of the cellular plasma membrane and their display on the surface of budding VLPs, thus providing a native conformation for downstream assays.

Parental insect Sf-9 and High Five cells were randomly tagged with GFP-fused Gag or M1 proteins and FACS enriched with cells tagged in genomic “hot-spots” supporting high expression. A linker including a Flp recognition target (FRT) site was used to allow posterior removal of the marker gene from the particle through cassette exchange. By confocal microscopy we could observe that Gag localizes preferentially at the plasma membrane whereas M1 disperses within the cell. Upon promoting Flp-mediated recombination in the tagging populations, cassette exchange was well succeeded, allowing the recovery of cells tagged in loci supporting FMCE. We have evaluated the capability of both core proteins as scaffolds to display GPCRs (e.g. beta-2 adrenergic receptor) and Influenza HA proteins. For the latter, we will present recent results on the feasibility of combining different bioprocess optimization strategies in order to improve cell line specific productivities.

Overall, modular insect cells platforms are being generated to be readily adaptable for production of a broad range of VLP-based vaccines as well as receptor display particles for drug screening or antibody discovery.

Keywords: Insect cell expression; Flipase-mediated cassette exchange; Pseudotyped HIV Gag-VLPs; Vaccines

O24 - Accelerating Recombinant Protein Availability in Stable Insect Cells Through a Novel Recombinase-based Targeting Strategy

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Efficient strategies for the production of recombinant proteins become crucial as the advances in the fields of vaccine development, drug-discovery and structural biology demand high yields of high-quality proteins. Stable cell lines are most suitable for recombinant protein production, enabling continuous expression and rising space for bioprocess optimization. To avoid clone-screening efforts related with traditional cell line development, gene targeted integration by recombinase mediated cassette exchange (RMCE) allows re-targeting of established genomic loci for expression of different proteins, enabling predictable expression patterns in cell culture. A tagging cassette flanked by a pair of incompatible Flipase (Flp) recombination target (FRT) sites harboring a reporter gene is randomly introduced into the cellular genome. Provided on a donor vector, an analogous cassette encoding the gene-of-interest can cleanly replace the former cassette, in a reaction mediated by Flp.

Herein we show results of an optimized Flp-mediated cassette exchange (Flp-RMCE) strategy for insect cell line development. A Flp-RMCE competent cassette was introduced into Sf9 and Hi5 cells by two rounds of cassette exchange followed by FACS, leading to stable and homogenous *gfp* reporter gene expression. Stable populations and clones of Sf9 and Hi5 cells were then compared for the expression of complex proteins after Flp-RMCE, including the *Human Cytomegalovirus* membrane glycoprotein B and Influenza virus like-particles.

Overall, we show that insect cell-Flp-RMCE factories represent a worthy alternative to traditional systems for producing complex recombinant proteins and we foresee that can tackle even the most challenging protein assemblies in the near future.

Keywords: Flp-RMCE technology, stable insect cell lines, Sf9 and High Five cells, virus like-particles

Poster Presentations

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P1 – Supercritical fluid extracts of *Arbutus unedo* distillate production residues. A valorisation approach

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The strawberry tree (*Arbutus unedo*) is a small (±4 m) evergreen plant belonging to the *Ericaceae* family and an endemic specie of the Mediterranean basin. Strawberry tree fruits in Portugal grow mostly wild and are mainly used to produce a distillated alcoholic beverage called “Aguardente de Medronho”. A fruit residue is obtained from the whole process that has no current use. In fact it is normally discarded. This residue still contains many compounds of interest from a bioactive point of view, namely lipophilic compounds such as fatty acids and terpenes. Supercritical fluid extractions figures out as an efficient and environmentally secure way to recover such compounds from that residue.

In the aim of the PhD programme of Sustainable Chemistry, this work intends to valorise this residue and integrate this valorisation in the downstream process of the beverage production facility, thus creating a biorefinery likewise facility. A set of experiments was performed using a factorial design (25-1) varying pressure, temperature, co-solvent (ethanol), flow rate and time of extraction in order to perform a screening to establish the best extraction conditions for bioactive fatty acids and terpenes recovery, using supercritical fluid extraction. An optimization design of experiments (central composite design) was then performed. These extracts were analysed by GC-MS and citotoxicity in Caco2 cell models of selected extracts was evaluated as well as antiproliferative effect in HT29 cells.

Keywords: *Arbutus unedo*, Supercritical fluid extraction, Biorefinery, Valorization, Sustainability

P2 – Functional characterization of two paralogous sporulation-specific proteases of *Clostridium difficile*

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Clostridium difficile is an obligate anaerobe intestinal pathogen causing diarrhoea and potentially lethal colitis. Spores produced by this organism allow its persistence and dissemination. Sporulation is a cell differentiation program in which two cells, the forespore and the mother cell, cooperate to form a spore. In *Bacillus subtilis*, a signalling protease produced in the forespore, SpoIVB, is essential for the activation of a pro-sigma factor, pro-σ^K, in the mother cell. However, this protease has a second, uncharacterized function in sporulation unrelated to the activation of pro-σ^K. Remarkably, in *C. difficile* σ^K is produced in active form, yet the genome codes for two developmentally-regulated *SpoIVB* paralogues. Aiming at deciphering the function of the two SpoIVB proteins of *C. difficile* we started by constructing in-frame deletion mutants. We show that the first mutant is producing 10 times less spores than the wild type strain, while the second mutant does not seem to produce heat resistant spores. Using phase contrast and fluorescence microscopy we show that the two mutants enter the sporulation pathways and form an asymmetric septum, but fail to complete engulfment of the forespore. This suggests that both *spoIVB* genes are required for engulfment completion and late gene expression during spore development in *C. difficile*. Work in progress aims at establishing the genetic and morphological requirements for expression of either gene, and the subcellular localization of the two SpoIVB proteins using the SNAPCd reporter.

Because of the central role of the spore in pathogenesis, and given their essentiality for sporulation, the two *C. difficile SpoIVB* paralogues may turn out to be good targets for the development of new anti-infective strategies.

Keywords: *Clostridium difficile*, spore development, cell type-specific gene expression, SNAPCd fusions, fluorescence microscopy.

P3 – Design and assembly of a prototype opto-electronic nose

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Microbial infections, especially when associated to antimicrobial resistant bacteria, constitute a healthcare burden worldwide [1]. Currently, conventional methods for detecting bacterial infections take at least 24-48h [2]. Since different bacteria release distinct volatile organic compounds (VOCs), as part of their metabolic activity, electronic noses have been explored to identify bacteria by the VOCs patterns [3]. The aim of this project is to develop an opto-electronic nose to identify bacteria at an early-stage and in a non-invasive manner. A prototype was designed and assembled, using sensing films that have unique stimuli-responsive properties when interacting with VOCs. The interaction between the VOCs and the sensing films, results in both optical and electrical changes which can be collected and processed. Several features can be extracted from the signals obtained, and though the use of pattern recognition methods, different VOCs can be identified. The optical sensor was validated, testing 13 different vapours of organic solvents. Several classification methods were applied and the accuracy of each one was evaluated. The predictive capacity for Support Vector Machine was 100%. Moreover, principal component analysis showed 13 clusters of VOCs clearly separated.

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Keywords: Bacteria, Electronic Nose, Principal Component Analysis, Volatile Organic Compounds

P4 – New Water Soluble Ir(III)-NHC Complexes for Waste Valorization

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Dyeing processes produce a large amount of wastewater rich in azo (-N=N-) dyes, which represents an environmental hazard. Degradation of azo dyes has been accomplished using an enzyme extracted from *Pseudomonas putida* (PpAzoR) [1]. From this enzymatic degradation aromatic amines are formed, which are important building blocks in several chemical processes. These amines can be valorized through chemical modifications like N-alkylation of amines with alcohols. Our aim is to develop a compatible organometallic complex to perform cooperative catalysis with the enzymatic system in order to valorize waste. N-alkylation of amines with alcohols has already been performed by several iridium based complexes but only few in water [2]. The development of water soluble metal-catalysts allows more sustainable chemical processes. Water solubility can be accomplished through the use of appropriate ligands like N-Heterocyclic Carbenes (NHCs) [3]. These are very versatile ligands and the metal-NHC bond is highly stable to hydrolysis, which is an attractive feature in coordination chemistry and catalysis. We report herein preliminary results of new water-soluble Cp*Ir(NHC)Cl_2 complexes in N-alkylation of amines with alcohols.

Acknowledgements: We acknowledge financial support from FCT through Research unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013), NMR Facility (RECI/BBB-BQB/0230/2012), and for grant PD/BD/109637/2015 (A. F) and consolidation contract IF/00346/2013 (B. R).

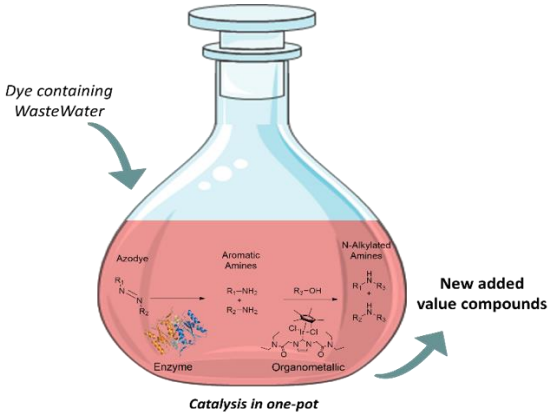

Fig. 1 - Cooperative catalysis in waste valourization

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Keywords: Organometallic, Catalysis, Waste Valourization, Iridium, amines

P5 – Identification of molecular regulators involved in the response to heat stress and suberization in *Quercus suber*

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Q. suber cork is a self-regenerating raw material with unique qualities and diverse range of applications, contributing to local economies and forest conservation. Despite cork oak ecological and economic impact, it is still a neglected species for molecular biology studies. Suberin, as the main component of cork cell walls, is the major determinant of the unique physical properties of cork. Suberization process is tightly-controlled by endogenous and exogenous factors such as environmental stimuli. At GPlantS Unit we study cork oak from two different perspectives: plant adaptation to stress and cork development. Here we report the identification and characterization of a putative Omega-3 fatty acid desaturase (QsO3FAD) and insights into the role of combined stresses on suberin biosynthesis and deposition.

QsO3FAD is down-regulated by heat shock and heat acclimation, suggesting a role in the regulation of membrane stability to withstand this stress. Yeast One-hybrid screenings targeting its promoter had found an interaction of a MYB transcription factor. Additionally, aiming to identify stress responsive regulators of suberin metabolism we are presently using *A. thaliana*. Suberin accumulation under control and non-lethal stress conditions are being monitored during early secondary growth in *A. thaliana* roots. The information gathered by this analysis will define critical timepoints for transcriptomic analysis, which will be used to identify novel players regulating suberin metabolism during secondary development. This knowledge will be further transferred to cork oak.

Keywords: *Quercus suber*, *Arabidopsis thaliana*, cork development, suberin

P6 – Beneficial effects of (poly)phenols in a rat model of hypertension: does microbiota play a role?

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It's been increasing the evidence that associate beneficial effects of a diet rich in (poly)phenols with a reduced risk of cardiovascular diseases. The biological response to (poly)phenols is greatly determined by their metabolization and further bioavailability. (Poly)phenol circulating metabolites are nowadays considered the bioactive molecules responsible for the health benefits.

Our goal was to identify the metabolic fate of (poly)phenol metabolites in a rat model of hypertension, as well as the influence of diet in the rat's gut microbiota.

A salt-sensitive rat model was used to develop hypertension pathology. Rats diet was supplemented with a berries mixture (blueberries, blackberries, raspberries, Portuguese crowberry and strawberry tree fruit) for 9 weeks. Rats were fed with different diets: Low Salt (LS), Low Salt and Berries (LSB), High Salt (HS) or High Salt and Berries (HSB). Urine, faeces and organs were processed and analysed by UPLC-MS/MS. Faeces were also processed for 16S rRNA Gene Sequencing.

Five rats in HS group died from stroke before the end of trial, while all LS, LSB and HSB survived. The increase in kidney weight index detected in HS was attenuated in HSB rats; moreover in HSB rats kidneys' s (poly)phenol metabolites were exclusively present. Differential urinary and faeces phenolic profile was also observed for HSB rats, that is in agreement with kidney results. Regarding gut microbiota, the presence of high amounts of salt and the presence of berries affected the abundance of the different phyla.

In conclusion, we accomplished to identify (poly)phenol metabolites present in the animals were it was observed a cardioprotective effect. Additionally we also disclosed the influence of the diet in modulating the gut microbiota. Further studies will be pursued to associate these alterations with the cardioprotection.

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Keywords: Rat model of hypertension, (poly)phenols, cardioprotection, metabolic fate, gut microbiota

P7 – Biofilm formation by *Clostridium difficile*

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Bacteria can grow attached to a surface forming multicellular communities surrounded by a self-produced extracellular matrix, composed of exopolysaccharides, proteins and nucleic acids. This structure is called biofilm. The importance of biofilm development by certain bacteria has been more recently recognized. One example is the anaerobic intestinal pathogen *Clostridium difficile*, a leading cause of healthcare-associated infections. It takes advantages of misbalances of the host microbiota to colonize and cause a spectrum of diseases. Spores produced by *C. difficile* allow its persistence and dissemination. *C. difficile* is also able to form biofilms both *in vivo* and *in vitro,* however the role of biofilms in disease is unknown. Therefore, although the persistence/recurrence of infection is attributed to spores, the involvement of biofilms, which also endow the bacteria with resistance to antimicrobials, cannot be ruled-out.

We started by stablishing the growth conditions that promote biofilm formation in liquid medium. We quantified with crystal violet the formation of biofilm in several media supplemented with glucose, bile salts, NaCl or glycine. With the aim of testing a possible correlation between epidemic strains and the ability to form biofilm, we quantified biofilm formation by a panel of *C. difficile* strains. No significant differences were observed between epidemic and non-epidemic strains. Finally, we show that, in contrast to what was observed in other species, sub-inhibitory concentrations of an antibiotic (imipenem) have no effect in promoting biofilm formation. In parallel, we are investigating the role of an endosporulation signature gene that recently was shown to be involved in biofilm formation in others sporeformers.

Keywords: Gut Microbiology, *Clostridium difficile*, biofilms, antibiotic resistance, crystal violet staining

P8 – Sustainable Strategies for Treatment of Water Contaminated with Persistent Pesticide Pollutants

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Agriculture practices have become increasingly intensive, in order to provide food at reasonable prices for an increasing number of people. The presence of pesticides in the water sources of public drinking water systems can be linked to a range of human and environmental health effects including cancer, neurological and reproductive diseases, while decreasing the biodiversity [1,2]. There are several methods for treatment of wastewater but none of them directly deals with pesticides removal. In this context, this work addresses development of novel efficient sustainable strategies, namely adsorption and liquid-liquid extraction using a novel class of compounds.

There has been an increasing search for new adsorbents and processes that can be used to replace some of the common adsorbents. Ionic liquids (ILs) present excellent solvating qualities, which can be tuned by varying the chemical structures in the cation or in the anions, and, consequently, their properties can be modified according to a specific application [3]. Polymeric ionic liquids (PILs) are a new class of polymeric materials that combine some of the unique properties of ILs and the intrinsic polymer features. In this work, the removal of pesticides pollutants, namely neonicotinoids, from aqueous environments using deep eutectic solvents and PILs will be evaluated. In particular, adsorption and liquid-liquid extraction techniques, usually used in these kinds of water treatments will be tested in the extraction of several pollutants.

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Keywords: Ionic Liquids, Deep Eutectic Solvents, Adsorption, Liquid-liquid extraction, Water treatment

P9 – The role of *Streptococcus pneumoniae* RNase R in ribosome assembly and translation

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Ribosomes are macromolecular machines that translate mRNA into functional proteins. In bacteria, the 70S ribosome is composed of two subunits, a small 30S subunit and a large 50S subunit. Each subunit is composed of ribosomal RNA and ribosomal proteins. A proper and coordinated assembly of these players is crucial to form an active ribosomal particle.

Ribonucleases (RNases) are enzymes that ensure maturation, degradation and quality control of RNA thus, contributing to the maintenance of the optimal amount of each transcript in the cells.

Not surprisingly, RNases have been associated with the assembly process acting as processing enzymes.

Escherichia coli RNB family of enzymes is present in all domains of life and includes RNase R, RNase II and the eukaryotic Rrp44/Dis3, Dis3L1 and Dis3L2 proteins. In *Streptococcus pneumoniae* only RNase R was identified. RNase R, encoded by the *rnr* gene, hydrolyzes RNAs starting from the 3' end. RNase R level is increased in several stress conditions such as heat shock, stationary phase or cold shock, conditions in which most of the proteins translation is blocked.

Here, we investigated the role of RNase R in translation by comparing the wild type strain with an *rnr* mutant strain. For this purpose, we compared the ribosomal profile between the two strains using sucrose gradient polysome separation and Western blots. We have also performed Northern blots analysis of transcripts involved in translation and ribosome assembly.

In this study we show that RNase R interacts with ribosomes mostly with the 30S subunit. Moreover, in the absence of this enzyme we have observed a decrease in the amount of the 70S ribosomal subunit, concomitantly with the increase of 50S subunit. RNase R seems also to be involved in the regulation of other RNases responsible for the processing of ribosomal RNA.

This study highlights the importance of *S. pneumoniae* RNase R in translation.

Keywords: Bacteria, Degradation, Translation, Ribosome

P10 – Forecasting threats of pollution in forest soils: changes in the functional diversity of fungal communities as case study

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Aims: Unravel how the functional biodiversity of belowground fungi in forest soils changes during the mitigation of an atmospherically relevant pollutant -pentachlorophenol (PCP), by using experimental simulations that may forecast pollution risks in real ecosystems.

Methods and results: Previous studies led to discovery of a PCP mitigation pathway used by belowground fungi in forest soils; its prevalence may be correlated with PCP atmospheric pollution and/or misuse. Using here similar experimental simulations (submerged cultivation) we evaluated the community differential response during exposure to 38 µM of PCP compared to control conditions. At the 3rd, 5th, 7th and 10th day of exposure we analysed the extracellular and mycelial sub-fractions, characterising both PCP degradation by the community and its taxonomic and functional diversity.

Next Generation Sequencing of mycelia collected at the 3rd and the 10th day of incubation (corresponding to PCP degradation levels of 1% and 70%, respectively) showed that PCP exposure dramatically altered the taxonomic diversity of the community. Fungal biomass was higher in control conditions, but between the 7th and 10th day of exposure the growth rate surpassed that of the control, suggesting recovery when substantial PCP mitigation is reached. In both sub-fractions and for all time points, a diversity of PCP related metabolites, particularly of downstream products of its degradation pathway (1) was identified (UHPLC-HRMS), consistent with its mineralization.

Conclusions: Our experimental approach is successfully revealing that even sub-lethal concentrations of PCP lead to specialisation of the fungal community, altering its taxonomic and functional profile.

Significance of study: Sub-lethal pollution may promote shifts/losses in the taxonomic and/or functional diversity of the belowground fungal communities impacting critical ecosystem services; study significance increases when sub-lethal yet chronic pollution is considered.

Keywords: Fungi, Next Generation Sequencing, Environmental Impact, Pentachlorophenol, MS-based Metabolomics

P11 – Insulin degrading enzyme knockout mice under high fat diet shows a worst glucose intolerance prognosis: preliminary results

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After secretion by pancreas, liver can remove 60 to 40% of the insulin that reach portal vein, a process called Insulin Clearance (IC) with great matter for the determination of peripheral levels of insulin and insulin sensibility. One of the key enzymes of this process is Insulin degrading enzyme (IDE). IDE-Knockout (IDE-KO) model is naturally hyperinsulinemic and develop disturbances in glucose tolerance and insulin resistance in an age dependent manner. We hypothesized that interaction of genetic predisposition to IC impairment with diet factors are an early component of the diabetogenic etiology complex. To test this hypothesis, wild type (WT) and IDE-KO C57BL/6 6 week old mouse were submitted to a high fat diet (HFD). Oral glucose tolerance test (OGTT) was performed after 4, 8 and 12 weeks of HFD. Glucose levels were evaluated at fasting, 15, 30, 60 and 120 minutes after an oral injection of 1.5g of glucose/body weight. Blood samples were collected during OGTT for measurement of insulin and c-peptide levels and evaluation of the IC (c-peptide/insulin ratio). No significant alterations were observed in terms of body weight and food intake. After 4 weeks of HFD, IDE-KO animals already presented alterations in terms of glucose tolerance as evidenced by the increase in the area under the curve (AUC) during OGTT in comparison with wild type (AUC IDE-KO: 27890±518.5 vs IDE WT: 21370±1190; $P<0.01$). Furthermore, glucose levels two hours after a glucose challenge was higher in the IDE-KO model in comparison with WT (84±8.9 vs 104±4.5mg/dL; WT vs KO respectively; $P<0.05$). Moreover, glucose intolerance was progressively impaired at 8 and 12 weeks of HFD. IC in the WT mouse showed a tendency to be higher than IDE-KO during the OGTT, since IDE were not expressed in this model. Based on these observations, we conclude that diet alterations associated with a genetic predisposition to IC impairment can anticipate the appearance of a glucose intolerant phenotype.

Keywords: Knockout mice, Insulin Clearance, Insulin Resistance, Glucose Intolerance

P12 - Role of an endosporulation signature gene in the assembly of the spore surface in *Clostridium difficile*

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Clostridium difficile is a leading cause of nosocomial infections causing potentially lethal intestinal diseases. The key agent for transmission are the spores formed by this anaerobic bacterium which germinate in the colon in response to bile salts. Despite the importance of spores in the infectious cycle, our knowledge of the molecular mechanisms underlying spore development is still scarce. Proper morphogenesis of the spore surface is largely governed by RNA polymerase sigma factor σ^K, which drives production of key components of the coat and exosporium layers that contribute to spore resistance and mediate the environmental interactions of the spore. Recent work shows that the timing of σ^K activation is crucial for proper assembly of the surface layers. We aimed at identifying important σ^K-dependent protein components of the coat/exosporium that may prove to be novel targets for preventing spore germination and/or binding to host cells.

In *Bacillus subtilis, yabG* is the only sporulation-specific gene in the σ^K regulon that contributes to a genomic signature of endosporulation, hinting at an important role for YabG in the assembly of the spore surface layers. The *yabG* is conserved in *C. difficile* and in most Clostridia but its expression has not been analysed in detail. We have constructed *yabG-SNAPCd* transcriptional and translational fusions to monitor the time and cell type-specific expression of *yabG* as well as the subcellular localization of the protein during sporulation. Fluorescence microscopy reveals that expression of P_{yabG}-SNAPCd is confined to the mother cell and detected at a late stage of sporulation when the spores become phase bright, consistent with σ^K control. Moreover, we show that the fusion protein accumulates in sporangia carrying phase bright spores and localizes in a ring-like pattern around free spores. *In toto*, the results suggest that *yabG* is a σ^K-dependent gene coding for a component of the spore coat/exosporium of *C. difficile.*

Keywords: *Clostridium difficile,* Spore development, Spore coat, *SNAPCd* transcriptional, Translational fusions, Fluorescence microscopy

P13 - Characterization and activity of Alternative Complex III from *Rhodothermus marinus*

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Alternative complexes III (ACIII), is central to several respiratory chains due to its quinol:cytochrome c oxidoreductase activity. ACIII was identified for the first time in *Rhodothermus marinus* as functional substitute of cytochrome bc1/b6f complexes [1, 2]. ACIII is a multisubunit complex with four integral transmembrane subunits (ActC, D, F and G) and three peripheral subunits a pentaheme and a monoheme cytochrome (ActA and ActE, respectively) and a large [Fe-S] clusters containing subunit (ActB). ActA and ActE are facing the periplasm, as observed for all cytochromes c, while the orientation of ActB subunit is unclear [3]. We aimed to functionally and structurally characterize ACIII, for which we used several complementary biochemical and biophysical approaches including cryo-electron microscopy, electrochemistry, enzymatic assays and 1H-NMR spectroscopy. We observe ActB subunit is facing the periplasm and that ACIII can reduce the cytochrome c domain of the caa3 oxidase as well as two other electron shuttles, HiPIP and soluble cytochrome c. Moreover, preliminary electrochemical studies suggest ACIII is contributing to the establishing of the membrane potential. All these findings strongly contribute to the unraveling of the operative mechanism of ACIII.

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Keywords: *Rhodothermus marinus*, 1H-NMR spectroscopy, cryo-electron microscopy electrochemistry, enzymatic assays, quinol:cytochrome c oxidoreductase activity

P14 - Crystal structure of GatD from *Staphylococcus aureus* reveals molecular determinants in peptidoglycan amidation

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Peptidoglycan biosynthesis relies in complex molecular machinery where dedicated enzymes are involved in several sequential synthetic steps. Peptidoglycan amidation is a crucial secondary modification for the survival and pathogenicity of bacteria. In *Staphylococcus aureus*, the recently discovered bi-enzymatic complex MurT-GatD is the liable player for the reaction. GatD is a glutamine amidotransferase responsible for ammonia transfer from free glutamine to MurT which will further amidate the peptidoglycan precursor lipid II.

In this work we report the crystal structure of *Sa*GatD solved at 1.9 Å resolution. The active site comprises residues C94 and H189, highly conserved in all class I glutamine amidotransferases. The glutamate residue that normally forms the catalytic triad in this class of enzymes is absent in the *Sa*GatD structure, indicating the presence of a catalytic dyad, instead. A glutamine molecule is found at the surface of the protein, close to the active site funnel and establishes hydrogen bonds with R128, suggesting that this arginine might be important for sequestering the substrate towards the catalytic site. Important structural features in deamidation reaction mechanism of glutamine as the oxyanion hole and the nucleophilic elbow, present in well characterized glutamine amidotransferases, are also observed in the structure here reported. Sequence analysis suggests that *Sa*GatD is similar to enzymes involved in cobalamin biosynthesis, where, until now, no structural information was available.

The structure of *Sa*GatD here reported will provide significant insights into the molecular basis of the so far undisclosed mechanism of peptidoglycan amidation in *S. aureus*. Considering the important role of these proteins in this organism and their ubiquitous presence within Gram-positive bacteria, its structural characterization may enable the development of new drugs or combining therapeutics to fight bacterial infections.

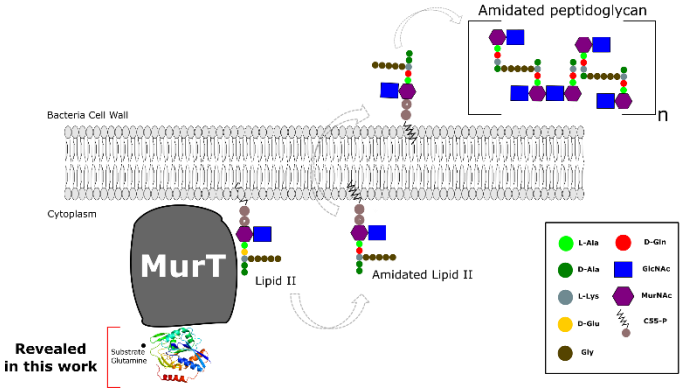

Figure 1. Peptidoglycan amidation catalysed by MurT-GatD protein complex

Keywords: *Staphylococcus aureus*, X-ray Crystallography, Multi-drug resistant bacteria, Peptidoglycan biosynthesis, Glutamine amidotransferase

P15 - Prospecting *Olea europaea* in Portugal: establishment of a base collection for the development of molecular tools to improve the "Galega" variety

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The cultivated olive tree (*Olea europaea* L. subsp. *europaea*) is a perennial plant widely cultivated throughout the world, having a high economic relevance to Portugal. The main national variety, "Galega", is of major importance due to the excellent olive oil that results from it, associated with several protected designations of origin (PDO). However, its production has suffered an intense reduction and subsequent replacement by foreign varieties, since the latter are more suitable for semi-intensive production systems, but giving rise to an oil of inferior quality.

Accordingly, it is essential to improve the "Galega" variety, starting by characterizing the existing variability, using genetic and agro-morphological information. This preliminary characterization will be fundamental to select the most interesting genotypes.

To assess the diversity of the "Galega" cultivar used in Portugal, a base collection was established with genotypes prospected from the different traditional regions of olive production.

These prospections were performed tacking into consideration the age of the olive trees (preferentially centenary) and orchard information provided by the farmers, resulting in a selection of a total of three hundred and thirteen representative genotypes/clones. This material will be the basis for the development of "Galega" haplotypes. These actions will allow future genetic studies in ideal conditions for the development of essential molecular tools to improve the "Galega" and correctly select the most interesting genotypes for production.

Keywords: *Olea europaea;* Genetic variability; National prospection; Agro-morphological characters

P16 - Newly identified autoinducer-2 receptors in the *Clostridium* genus

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Autoinducer-2 (AI-2) is a quorum sensing signalling molecule involved in inter-species communication that is produced and recognized by a wide variety of bacteria [1]. Many bacteria use AI-2 mediated quorum sensing to regulate important bacterial population wide behaviours ranging from biofilm formation to antibiotic production. However, only two classes of protein receptors for this molecule have been identified: LuxP, which has only been found in the *Vibrionales* and LsrB which has been described in members of the *Entereobacteriaceae*, *Rhizobiaceae* and *Bacillaceae* families [2-4]. Here we show the presence of an LsrB-like receptor in *Clostridium saccharobutylicum*. We identified six candidate receptors in species belonging to the *Firmicutes* phylum through bioinformatics. By employing biochemical binding assays and crystallography we confirmed that the AI-2 receptor present in *C. saccharobutylicum* is able to bind the non-borated form of AI-2 and has high structural homology previously identified LsrBs. However, this LsrB-like receptor differs in its AI-2 binding site from previously characterized; two of the 6 amino acid residues that contact AI-2 are not conserved in the *C. saccharobutylicum* receptor. This is the first known case of binding site variance without loss of functionality. Our observations provide the first report of the presence of AI-2 receptors in the *Clostridiaceae* family, which includes important human pathogens, as well as beneficial members of the microbiota. Additionally, our results demonstrate that the binding site of the LsrB family of receptors is more diverse than previously thought. These findings will be important for the identification of novel AI-2 receptors in other family of organisms where AI-2 receptors have not been identified yet.

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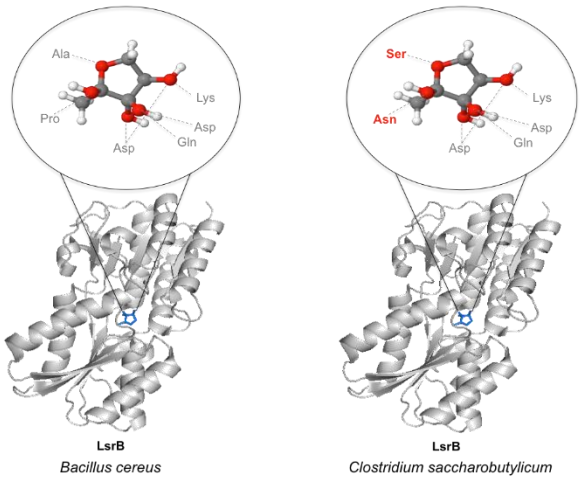

Fig. 1 - Representation of the LsrB receptors from *Bacillus cereus* and *Clostridium saccharobutylicum*. The six amino acid residues from the binding site that contact with AI-2 are evidenced.

Keywords: *Clostridium saccharobutylicum*; crystallography; quorum sensing; AI-2 receptors; LsrB

P17 – Studying the link between C4 metabolism and growth in maize leaves

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Maize has high economic value since it is one of the most produced cereal crops that is grown widely throughout the world and serves a multitude of purposes. Maize is a C4 plant and plants with C4 metabolism have a higher growth rate than C3 plants. However, the link between leaf growth and C4 photosynthesis remains poorly understood. The aim of this project is to gain a better insight into how leaf growth and C4 metabolism are regulated during leaf development and to identify genes that are involved in the regulation of both growth and photosynthesis in maize.

Genetic perturbations that increase leaf growth and environmental perturbations, such as mild drought, that lower the growth rate, are being used for detailed phenotypic analysis, incorporating growth analysis and physiological parameters to study the connection between C4 metabolism and growth.

Numerous genome-wide transcriptome studies are available that studied either growth or C4 metabolism in the maize leaf. An algorithm was developed to mine these transcriptome datasets and to select genes that might be involved in the regulation of both growth and C4 metabolism. For four of these genes, overexpression and knock out maize lines, through the CRIPSR-CAS9 technology are currently being generated. The resulting transgenic lines will be phenotyped for growth and photosynthesis parameters.

A better understanding of the interplay between C4 metabolism and growth might allow novel strategies for crop yield improvement.

Keywords: Maize, C4 Photosynthesis, Leaf Growth

P18 – Unravelling seed development in the common bean (*Phaseolus vulgaris*) integrating transcriptomics and proteomics data

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Common bean (*Phaseolus vulgaris*) is an important staple food worldwide. We conducted a proteomic and transcriptomic analysis to unravel the molecular mechanisms of P. vulgaris seed development (SD).

A gel-free proteomic assay (LC-MS/MS) was done on seeds at 10, 20, 30 and 40 days after anthesis, spanning from late embryogenesis until desiccation. At the early SD stage an accumulation of proteins related with protein metabolism, glycolysis, TCA, stress and nucleic acid metabolism was observed, reflecting an extensive metabolic activity. At mid-SD the accumulation of storage, signaling, starch synthesis and cell wall-related proteins stood out. In the later stages, an increase in proteins related to redox, and to a lesser extent protein degradation/modification/folding and nucleic acid metabolisms reflect that seed desiccation-resistance mechanisms were activated. Clues on maintenance of genome integrity during SD were unveiled, such as proteins involved in chromatin remodeling (histone H2A) and DNA repair (proliferating cell nuclear antigen). In parallel, we also investigated changes in gene expression profiles during SD. For this purpose, we performed a Massive Analysis of cDNA Ends (MACE) at the same time points.

The integration of information from both analyses enhances our knowledge on the SD molecular mechanisms. This knowledge may be used in the design and selection of common bean seeds with desired quality traits.

Keywords: Plants, Proteomics, Transcriptomics, Seed Development, *Phaseolus vulgaris*

P19 – New iron catalysts for environmentally friendly oxidation reactions

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The low price, high abundance, and non-toxicity of iron, along with the great popularity of *N*-heterocyclic carbine (NHC) ligands in catalysis have motivated the growing interest in developing the chemistry of Fe-NHC [1]. Our group has contributed to this area of research with the development of piano-stool iron(II)-NHC complexes, and their application as efficient catalysts for the reduction of functional groups through hydrosilylation and hydrogen transfer processes [2]. Herein, we describe the synthesis and characterisation of iron complexes containing the "Fe-bis-NHC" fragment combined with the ancillary ligands 2,2-bipyridine (bipy) and 1,10-phenanthroline (phen), and their reactivity towards oxidation processes. The complex [Fe(bis-NHC)(bipy)I_2] (Fig 1) resulted to be an effective catalyst for the oxidation of 1-phenylethanol to acetophenone, with tert-butylhydroperoxide under neat conditions at 80°C.The scope of the reaction and the influence of different solvents in the performance of the catalyst will be discussed. Crystallisation of 1 by slow diffusion of Et_2O into MeCN solutions afforded single crystals of [Fe(bis-NHC)$(bipy)_2$]I_2; its structure has been determined by X-ray diffraction studies [3].

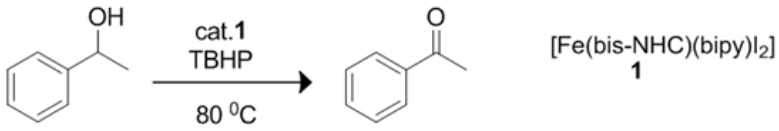

Figure 1: **Oxidation of alcohols in neat conditions with TBHP using the iron catalyst Fe(bis-NHC)(bipy)I_2.**

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Keywords: Organometallic Chemistry, Homogeneous Catalysis, Catalysis and synthesis, To develop Fe-NHC catalysts for catalytic oxidation process

P20 – Production of Oncolytic Adenovirus and Human Mesenchymal Stem Cells in a Single-Use, Vertical-Wheel Bioreactor System: Impact of Bioreactor Design on Performance of Microcarrier-Based Cell Culture Processes.

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Anchorage-dependent cell (ADC) cultures are used to produce viral vectors and virus-based vaccines as well as for cell therapies and tissue engineering applications. Currently, these applications rely on planar technologies (PT) for the generation of biological products. However, as new cell therapy product candidates move from clinical trials to potential commercialization, PT have proven to be inadequate to meet large scale manufacturing demand. Therefore, new scalable platform for culturing ADC at high cell volumetric concentrations is urgently needed. A promising solution is to grow cells on microcarriers in single-use bioreactors. Toward this goal, a novel bioreactor system using an innovative Vertical-Wheel technology (PBS) was evaluated for its potential to support scalable cell culture. Two anchorage-dependent human cells were used: human lung carcinoma cells (A549) and human bone marrow-derived mesenchymal stem cells (hMSC). Key hydrodynamic parameters such as power input, mixing time, Kolmogorov length scale, and shear stress were estimated. The performance of PBS was then evaluated for A549 cell growth and adenovirus production, and for hMSC expansion. Regarding the first cell model, higher cell growth and infectious viruses per cell were achieved when compared with stirred tank bioreactors (ST). For the hMSC model, higher percentages of proliferative cells could be reached in the PBS compared with ST though no significant differences in the cell volumetric concentration were observed. Noteworthy, hMSC population generated in the PBS showed a significantly lower percentage of apoptotic cells and reduced levels of HLA-DR positive cells. Overall, these results showed that process transfer and scale-up from ST to PBS was successfully carried out for two cell culture models. Ultimately, the data herein generated demonstrate the potential of PBS as a new scalable biomanufacturing platform for microcarrier-based cell cultures of complex biopharmaceuticals.

Keywords: Anchorage-dependent Cell Cultures, Scalability, Microcarriers, Single-use Bioreactor, Vertical-wheel Bioreactor

P21 – Unravelling the molecular mechanisms of a successful graft in grapevine

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The aim of my PhD work is to understand the molecular mechanisms controlling the success of grafting in *Vitis spp*. For this, we are studying the transcriptional and post-transcriptional regulatory mechanisms in two scion/rootstocks systems with different levels of graft union success. As plant material we used two clones of Touriga Nacional (TN) grafted the rootstock 110R, TN21 and TN112 that proved to have, on the field, different levels of graft success. Total RNA was extracted at the graft union, in two time points of the developing stage, 21 (callusing stage) and 80 days after grafting (rooting stage). MACE (Massive Analysis of cDNA Ends) and microRNAs (miRNAs) sequencing were used to produce transcriptomic data. A list of differentially expressed transcription factors (TFs) and miRNAs involved in the vascular tissue development was identified and analysed by digital PCR and quantitative PCR, respectively. The major results were found at rooting stage. At this stage, six of the eight tested TFs, VvWOX4, VvLBD4, VvERF3, VvERF1A, VvARF4 and VvARF6, were significantly more abundant in the more compatible graft when compared to the less compatible one.

At the post-transcriptional level, microRNAs highly present in the RNAseq libraries were analysed by qPCR. Two of these were Vvi-mir166 and Vvi-mi159. Vvi-miRNA166 targets, and negatively regulates, the expression of TFs of class III Homeodomain leucine zipper. These TFs are known to play an essential role on the regulation of the differentiation on the vascular system. Vvi-miRNA159c targets GAMYB TFs, proposed to be involved in gibberellin signalling pathway. Both miRNAs revealed to be differentially down regulated on the more compatible combination at the second time point.

These results suggest that there is a different temporal regulation of the graft union development with an upregulation the TFs associated with proliferation and differentiation of vascular tissues in the more compatible graft, at rooting stage.

Keywords: *Vitis vinifera*, Graft Success, Expression Profiling, Transcription Factors, microRNAs

P22 – A novel *Arabidopsis thaliana* membrane transporter regulates root growth in response to nutrient signals

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The Major Facilitator Superfamily (MFS) of membrane transporters remains barely studied despite being ubiquitous in living organisms and representing the second largest group of transporters on Earth. Our lab's previous work on the functional characterization of plant MFS transporters has revealed not only key roles in the response to environmental signals, but also striking examples of the biological relevance of alternative splicing - a key posttranscriptional mechanism to generate proteome diversity and regulate gene expression - in plant systems. During my functional analysis of *Arabidopsis thaliana* genes encoding uncharacterized membrane transporters of the MFS, I have found a novel MFS carrier, which we have provisionally named MFS17, that regulates growth of the primary root in response to nutrient signals. Indeed, upon isolation of a loss-of-function mutant for this gene, our phenotypical analyses revealed that mutant seedlings for MFS17 display a longer primary root than wild-type plants, with this difference in root length being enhanced when seedlings are grown in the presence of sugars. Interestingly, we have demonstrated that the MFS17 gene generates two alternative transcripts, the shorter of which is not yet annotated and potentially gives rise to a truncated protein. The observed phenotype appears to be due exclusively to the absence of the longer MFS17 transcript, as the mutant expresses wild-type levels of the shorter mRNA. I am currently working on the isolation of a mutant allele where expression of both MFS17 transcripts is impaired to further elucidate the in vivo roles of this transporter-encoding gene and the physiological significance of its alternative splicing.

Keywords: *Arabidopsis thaliana*, Nutrient Signalling, Root, MFS Transporter

P23 – Mimicking a Heart Attack in the Lab: Unveiling Human Cardiac Stem Cells Role In Myocardial Ischemia-Reperfusion Injury

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After an Acute Myocardial Infarction (AMI), Ischemia-Reperfusion (I/R) injury is responsible for a critical decrease in the number of viable cardiomyocytes (hCMs). Human myocardium harbors a population of endogenous cardiac stem cells (hCSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine molecular crosstalk between hCSCs and hCMs in stress.

Our work aims at setting up the first in vitro human I/R injury model in order to decipher the role of hCSCs and correspondent cross talk between hCSCs and hCMs upon AMI using proteomic tools.

Human CSCs, hCMs monocultures and co-cultures were established using human donor derived CSCs and hCMs derived from human induced pluripotent stem cells at different maturation stages (hiPSC-CMs). Ischemia was mimicked by substituting growth media by Ischemia Mimetic Solution and placing the cells at 0% O_2 for 5 hours. In the reperfusion step, cells were placed back in their physiological culture conditions (3% O_2). The effect of I/R injury in hCSCs was accessed by total proteome analysis at different time points by LC-MS. Growth factor secretion, cells' viability, as well as hCSC proliferation was also accessed in both mono- and co-culture systems. More than 2000 proteins were identified: proteins associated with mitochondrial dysfunction and oxidative stress response were identified in hCSCs exposed to injury. Important features of I/R injury were successfully captured, namely hCSC proliferation activation upon insult, increase in HGF secretion, and the protective role of hCSCs on hiPSC-CMs. The maturation stage of hiPSC-CM showed to be of high relevance in the response to injury. This system will allow further understanding on the molecular landscape of the myocardium during AMI, namely regarding hCSC regenerative response and hCM survival. The knowledge gained in this work will potentiate the development of novel therapies for myocardium regeneration.

Keywords: Human Cell Model; Proteomics; Ischemia/Reperfusion Injury; Myocardial Infarction, Human Cardiac Stem Cells.

P24 – Fluorinated Ionic Liquids as Novel Drug Delivery Systems for Therapeutic Proteins: Lysozyme case study

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In the past decades, the attractiveness of the study of therapeutic proteins has increased dramatically [1,2]. To overcome the major problems related to the use of proteins for therapeutics, different strategies have been developed. These new strategies include, among others, protection by encapsulation [1]. The encapsulation can be made through the development of novel drug delivery systems, avoiding the traditional problems of administration and maintaining the therapeutic levels of proteins without side effects [3].

The biological inertness of some fluorinated ionic liquids and their great surfactant power [4,5] make them even more promising than other ionic liquids. They may be used to form micelles for a nano-scale delivery system that will surround the proteins and protect them, improving their therapeutic action on the target site.

The main purpose of this study is to evaluate the capability of fluorinated ionic liquids to be employed as drug delivery systems for therapeutic proteins. With this purpose, a series of different studies were performed with lysozyme and includes activity and stability assays, circular dichroism (CD), dynamic light scattering and microscopy studies.

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Keywords: Fluorinated Ionic Liquids, Drug Delivery Systems, Lysozyme, Activity, Stability, Structure, Encapsulation

P25 – Gold-nanoparticles for MDR1 silencing in DOX treated Colon Cancer Cells

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Many cancers develop resistance to chemotherapeutic agents, which become a major obstacle to effective chemotherapy. Multidrug resistance (MDR) mechanisms may rely on the up-regulation of membrane ATP-dependent efflux pumps that excrete drugs to the extracellular medium, decreasing their intracellular concentration [1]. One of most studied examples is P-glycoprotein (MDR1) whose overexpression is described in many cancers, including colorectal and hepatocellular carcinomas, leukemia and lymphoma, where it confers cross-resistance to a variety of cytotoxic agents [1]. Targeting MDR mechanisms with iRNA capable to effectively silencing gene expression, which sensitizes tumor cells to cytotoxic drugs. iRNA requires effective vectors for the silencing moieties (e.g. siRNA, hairpin ssDNA) that can sustain degradation in circulation, deliver them intracellularly with minimal toxicity on target cells [2,3].

Previously we reported that gold nanoparticles (AuNPs) functionalized with hairpin antisense ssDNA oligonucleotides have equivalent silencing capacity and cellular toxicity than lipofectamine vectorized siRNA [4,5].

In this work, we used AuNPs functionalized with hairpin antisense oligonucleotides directed at silencing MDR1 to increase sensitisation of colorectal carcinoma (HCT116) cells to doxorubicin. We believe such systems will pave the way for combinatory strategies to overcome MDR in the clinics.

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Keywords: Animal Cells, Genetherapy, Chemotherapy, Combinatory therapy, gold nanoparticles

P26 – Novel strategies for antibody purification through crystallization

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The monoclonal antibodies (mAbs) industry has the highest growth in the biopharmaceutical sector [1]. The current chromatographic approaches used in protein purification are not keeping pace with the increasing market demand, therefore new and more efficient processes are required. New approaches rely in Anything But Chromatography methodologies, going back to former techniques. Precipitation and crystallization are methodologies already implemented in the downstream processing of different high added therapeutic value proteins, as insulin or human serum albumin [2].

With recent and promising results in mAbs crystallization and precipitation [3,4], this project focuses in the development of a new purification method entitled Magnetic Crystallization. Presently, a high throughput screening to reveal the crystallization condition of different mAbs is being performed. Further along, the aim is to target specifically the crystallization of mAbs from complex media and to assess the process scale-up potential.

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Keywords: Proteins (mAbs), Crystallization, Purification, Downstream process, Biopharmaceutical

P27 – The RNA chaperone Hfq is involved in ribosome biogenesis

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Hfq is an RNA-binding protein widely spread among bacterial organisms with a central role in post-transcriptional control of gene expression. Hfq is mainly recognised for facilitating the base-pairing between small RNA and their cognate mRNA targets. Most sRNA have been shown to require Hfq for their function and stability.

We have unravelled a novel function of Hfq. For the first time, Hfq was found to be critical for ribosome biogenesis. This intricate multi-step process involves the production, maturation and assembly of its 55 components to originate functional 70S ribosomes. Cells lacking Hfq are defective in rRNA maturation and ribosome assembly. The *Δhfq* strain accumulates higher levels of the 17S rRNA precursor and exhibits an altered ribosome profile with lower amounts of active 70S particles. Moreover, upon *hfq* inactivation the translational rate decreases significantly and miscoding errors occur more frequently, affecting translation at a global level. Overall, we have found that cells growing without Hfq exhibit many hallmarks of ribosome biogenesis defects and consequently a general translational block.

This work expands the knowledge on one of the major post-transcriptional regulators, adding a new role to Hfq as a ribosome biogenesis auxiliary factor.

Keywords: Bacteria, Ribosome profiling, Northern blotting, Ribosome biogenesis, Hfq

P28 – Genome sequence analysis of invasive and carriage pneumococcal isolates obtained during invasive disease

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Streptococcus pneumoniae (pneumococcus) is a commensal in the human nasopharynx and a causative pathogen of invasive and non-invasive diseases. It is thought that disease is always preceded by carriage in the upper-respiratory tract. The polysaccharide capsule is the main virulence factor with >95 capsular types (serotypes) described until now.

The aim of this study is to identify genomic differences between pairs of invasive and carriage pneumococcal isolates, obtained from the same patient, diagnosed so far, with invasive pneumococcal disease. A total of 47 patients between 0-14 years old have been enrolled in the study. Invasive pneumococcal isolates were obtained from sterile sites and nasopharyngeal aspirates were performed to screen for presence of pneumococcal carriage. A qPCR targeting *lytA* gene was performed to all samples to detect pneumococci, and a fluorescence capillary electrophoresis based method was used to detect the serotype. DNA extraction of pairs of isolates obtained from the same patient with matching serotype (invasive and carriage) was performed with the DNeasy blood and tissue kit (Qiagen, Netherlands). Genomes were sequenced by the Illumina HiSeq 2000 platform. The paired-end reads were checked for quality, trimmed, and *de novo* assembled. The carriage genomes were annotated and variants were identified by mapping reads of the invasive isolate to the annotated carriage genome.

Pneumococci were detected in 31/47 nasopharyngeal aspirates, but only 15/31 samples had matching serotypes. Of these, pneumococcal isolates representing 4 matching pairs have been sequenced. Several non-synonymous mutations were detected in genes that may be related with pneumococcal adaptation during invasion, reflecting the transit of the pneumococcus between different niches inside human body but also the pathogenesis of this bacterium. Nonetheless, at this stage mutations need to be further confirmed and consequences on protein function are still to be elucidated.

Keywords: Bacteria, *Streptococcus pneumoniae*, whole genome sequencing, invasive and carriage

P29 – Genome-wide association study of common bean disease resistance in Portuguese germplasm

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Common bean (*Phaseolus vulgaris L.*) is the world's most important grain legume for human consumption. Portugal holds a very promising common bean germplasm, which results from more than five centuries of natural adaptation and farmer's mass selection in the country, not yet fully explored in breeding. Fusarium wilt (caused by the soilborne pathogenic fungus *Fusarium oxysporum f. sp. phaseoli*) is among the diseases that cause major yield losses in this crop.

In order to identify new sources of resistance, we characterized the resistance to fusarium wilt of a collection of 162 Portuguese common bean accessions. Evaluation took place under controlled growth chamber conditions, using a replicated incomplete randomized block design. Infection responses revealed great variability among the accessions with the identification of interesting sources of resistance. This collection was additionally screened with ~12000 molecular markers (SNPs from Illumina BeadChip and DArTseq arrays) uniformly distributed throughout the genome. A genome-wide association study, joining the fusarium wilt disease resistance levels with the genomic information, was performed using a mixed linear model approach accounting for population structure and familial relatedness.

The putative statistically significant associations found, together with the development of molecular tools (user-friendly markers for the detection of functional alleles), will contribute to a more effective selection of common bean genetic resources in future disease resistance breeding programs.

Keywords: Bean, *Phaseolus vulgaris*, fusarium wilt, GWAS, resistance

P30 – *Casuarina glauca* salt stress tolerance: insights from a mass spectrometry-based metabolomics approach

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Casuarina glauca is a model actinorhizal plant characterized by its ability to establish symbiosis with nitrogen-fixing *Frankia* bacteria. This plant species grows naturally in coastal zones and is able to thrive under extreme salinity environments. *C. glauca* tolerance to high salinity is associated to biochemical and physiological adjustments such as low tissue dehydration, osmotic adjustments, and high membrane integrity. Mass spectrometry (MS)-based plant metabolomics has emerged as a powerful tool to address biological questions related to plant environment and agriculture. The measurement of primary metabolites (e.g. sugars, amino and organic acids) involved in the regulation of plant developmental processes has contributed to better understand how plant metabolism readjusts in response to abiotic stresses. To date, there is almost no information on the the *C. glauca* metabolome.

In this PhD project, a modern metabolomics approach that combines two MS-based analytical platforms, namely LC-QIT-MSn target analysis and GC-TOF-MS metabolite profiling, is being applied to study the impact of salt stress in symbiotic and non-symbiotic *C. glauca* plants. Our most recent results, presented in this poster presentation, agree with those previously obtained from morpho-physiological analysis, and provide new knowledge on the primary metabolome of *C. glauca*, its symbiosis with *Frankia* Thr, and its metabolic readjustments under increasing salt concentrations.

Furthermore, the divergent metabolite responses particularly found in the amino acid metabolism suggest root and root nodule specific metabolite responses, and support the fact that from 200 mM NaCl upwards, symbiosis was turned off. Based on these results, a second independent biological experiment is currently ongoing to assess, at the physiological and metabolite levels, the performance of non-symbiotic *C. glauca* plants under a combined salt and heat stress.

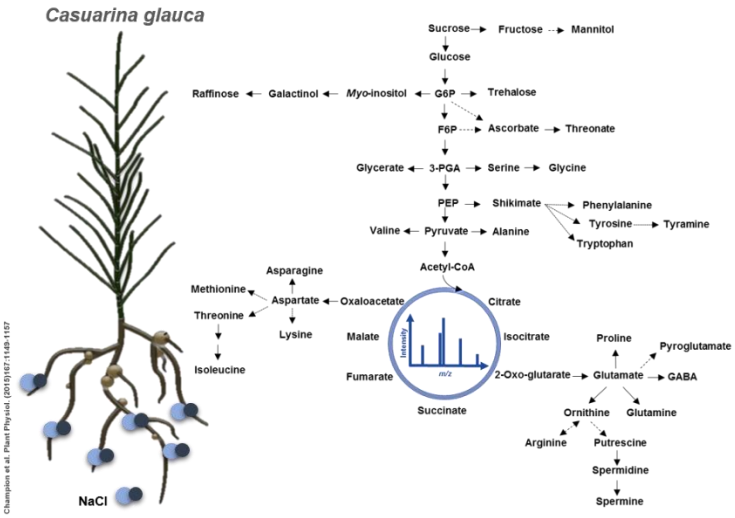

Fig.1 - Mass spectrometry (MS)-based metabolomics as a powerful tool to study the metabolic mechanisms underlying *Casuarina glauca* salt stress tolerance.

Keywords: *Casuarina glauca*, Symbiosis, Salt stress, Mass spectrometry, Plant metabolomics

P31 – Synthesis of prodrugs and chemical probes based on the structure of auto-inducer 2

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Quorum sensing is a cell-cell communication process used by bacteria to synchronize and regulate their group behaviours [1]. Autoinducer-2 (AI-2) is unique as it is produced and detected by many phylogenetically distinct bacteria [2,3]. Previous results have shown that AI-2 influence the balance of the major phyla in the gut after antibiotic treatment. Based on these results, we hypothesise that AI-2 plays an important role in controlling colonization and homeostasis of the gut microbiota contributing to the protective properties of these poly-species against pathogens [1].

Previous work has been developed in our group on the synthesis of (S)-4,5-dihydroxypentane-2,3-dione (DPD) (AI-2 precursor) and analogues starting from methyl glycolate in optically pure form [4,5]. Here we present the synthetic strategy to obtain new beta glycoside analogues of AI-2 (Fig. 1) that will function as prodrugs to deliver intact AI-2 to the gut, taking advantage of the enzymes that are only produced in the gut, beta-glycosidases. The anomeric selectivity of the glycosylation reaction between suitable DPD precursors and several different thioglycosides has been studied to obtain preferentially the beta anomer.

In parallel, we have been developing chemical probes based on the AI-2 structure using different synthetic strategies (Fig 1). These probes will allow the identification of new AI-2 receptors. Despite being widely expressed, at the moment only two classes of protein receptors for AI-2 have been identified, LuxP and LsrB [1,6].

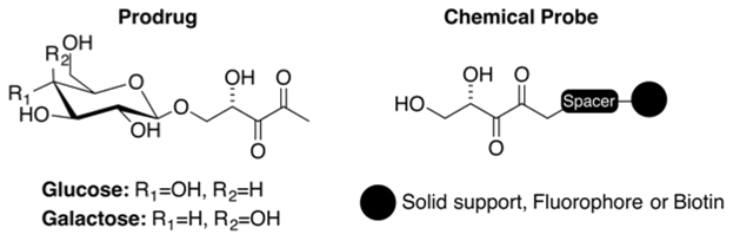

Fig.1 - Example of one proposed prodrug and a general example of a chemical probe.

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Keywords: Auto-inducer 2, Organic Synthesis, Quorum Sensing, Prodrugs, Chemical Probes

P32 – "Trick or treat?" - Evaluating carbohydrate recognition by the Human Gut Microbiome

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

Typically isolated microbial gut strains exhibit substrate-specific polysaccharide-utilization loci (PULs) encoding several putative glycoside hydrolases, carbohydrate-binding modules and starch utilization system D-like proteins [1]. The high number of newly sequenced gut bacteria and uncharacterized PULs is growing fast. As the information piles up, there's an urgent need to develop and apply high-throughput approaches to study these recognition systems.

Here, we present our integrative strategy that combines carbohydrate microarray technology [2,3] and X-Ray crystallography [4,5] to unravel glycan recognition by PULs architectural proteins from representative strains of the gut microbiota, *Bacteroides thetaiotaomicron* and *Bacteroides ovatus*, which have adapted to mammalian- or dietary-derived glycans, respectively. The designed strategy includes: sequence analysis and production of a recombinant-protein library (over 100 different protein domains); high-throughput screening analysis using carbohydrate microarrays comprising a diverse range of fungal-, plant- or mammalian-derived glycan sequences; and structural characterization of selected protein:oligosaccharide interactions using X-ray crystallography. This combined approach can be further applied to other microbial strains and will contribute to PULs functional analysis as well as to a broad understanding of the human microbiome metabolic capabilities.

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Keywords: *Bacteroides spp.*, Carbohydrate Microarrays, X-ray crystallography, Polysaccharide-utilization loci, Carbohydrate recognition

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