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10-12
October
2012

3rd ITQB PhD Students´ Meeting

the annual gathering of all the doctoral students enrolled at ITQB

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Book of Abstracts

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Dear participant,

Welcome to the 3rd ITQB PhD Students' Meeting!

This year, despite the financial difficulties, we hope we have kept the quality of previous meetings.
We believe that the ITQB PhD Students' Meeting is a unique opportunity for all generations of ITQB scientists to get together and we expect to see the scientific enthusiasm thriving during these three days. This year we decided to organize the oral and posters presentations by **thematic sessions** and to invite young doctorates to chair these sessions with us in order to take the scientific debate one step further. We also have **three poster sessions**, giving the participants more time to discuss their work. During this meeting 45 students will present their work.

To broaden our horizons, we have invited three keynote speakers from outside ITQB, all from a successful generation of scientists in Portugal, working in biological areas distinct from those existing in ITQB: **Francisco Dionísio** from **FCUL, Luísa Figueiredo** from **IMM** and **Arsénio Fialho** from **IST.**

To conclude our work program, a burst of energy will be brought to us by **Miguel Gonçalves**, a young psychologist who made his own original career path.

Finally we would like you all to join us at the **closing dinner**, kindly offered by **Câmara Municipal de Oeiras**, where we will deliver the awards for best oral and best poster presentation.

We are deeply grateful to all the people that have contributed to the organization of this event.

We hope you all enjoy this year's meeting!

The organizing committee:

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Keynote speakers

Interactive Session

Keynote Speakers

Francisco Dionísio, FCUL

Francisco is an Assistant Professor (Professor Auxiliar) at the "Universidade de Lisboa" and the head of the Evolutionary Ecology of Microorganisms group at the "Faculdade de Ciências da Universidade de Lisboa". His group is doing research mostly in evolutionary ecology of microorganisms, with a focus on bacteria, viruses and plasmids. Their approach involves both laboratory work and theoretical research. They study cooperation, competition, spite, and sex among bacterial cells, and their impact on pathogenicity and antibiotic resistance. Francisco concluded his PhD in Microbiology at the "Université de Paris VII" in 2000 and made a Post-Doc in David Haig group at Harvard University in 2005-2006. He is author of a chapter in the book 'Horizontal Gene Transfer in Microorganisms' and he has even published his own book about genetic conflicts – "Uma Tampa para cada Tacho".

Luísa Figueiredo, IMM

Luísa received her B.Sc. degree in Biochemistry from "Universidade do Porto" and her Ph.D. degree in Molecular Parasitology from "Institut Pasteur, France and Universidade do Porto" in 1997 and 2002, respectively. From 2003 to 2009 she was a Postdoctoral researcher at "The Rockefeller University, New York, USA". Since 2010, Luísa is the group leader of the Parasite Molecular Genetics Unit at the "Instituto de Medicina Molecular" in Lisbon, and became a leading expert in the parasite that causes African sleeping sickness - Trypanosoma brucei. Currently, she is focused in identifying the chromatin factors that activate or silence the different VSG genes to use them as therapeutic targets for the disease. For her efforts in this area, she already received several awards, including the 2010 Crioestaminal Award and the 2012 HHMI International Early Career Scientist.

Arsénio Fialho, IST

Arsénio M. Fialho received his B.S. degree in Biology from "Faculdade de Ciências da Universidade de Lisboa" and his Ph.D. degree in Biotechnology from "Instituto Superior Técnico (IST) da Universidade Técnica de Lisboa, in 1986 and 1996, respectively. Since 2004, he has been an Associate Professor at the Chemical and Biological Engineering Department, IST, and a Researcher Member of the Centre for Biological and Chemical Engineering, Institute of Bioengineering and Biotechnology. Recently, he has also received a Habilitation in Biotechnology at IST. His research interests are focused on the biosynthesis and role of bacterial exopolysaccharides, particularly, the commercial gellan gum from Sphingomonas elodea and the exopolysaccharide produced by Burkholderia cepacia isolates from cystic fibrosis patients. His current scientific interests are also centered on the study of bacterial proteins, such as cupredoxins, as novel promiscuous drug candidates with anticancer, antiparasitic, and anti-HIV activities. Complementarily, he has been deeply interested in bioinformatic and comparative analysis of Burkholderia genomes. He is the author of more than 60 papers in peer-reviewed scientific journals, 8 U.S. patents, several book chapters and two books entitled "O Mundo do RNA: Novos Desafios e Perspectivas Futuras" and "Emerging Cancer Therapy: Microbial Approaches and Biotechnological tools".

Interactive Session

Miguel Gonçalves, Spark Agency

Miguel Gonçalves is an atypical psychologist, a creative with childish imagination. He founded Spark Agency, a creativity agency specialized in design thinking and acceleration ecosystems and has created several frameworks that are redesigning the interface between people and companies:

So Pitch – Event that brings together a new generation of companies with people who want to work.

Udini – Intended to be the best mentoring network in Portugal, brings together CEOs and businessmen and people who want to launch their business ideas.

SIM, Ideias Para Portugal – Idea Generation framework focused on the problems of Portugal in 5 axis: Academy/Market; Employment; Exporting; Future materials; Tourism.

SiliconCard – A mobile app designed to empower networking in events, conferences and group activities. Launched in July, it has reached the top of Portuguese apps. siliconcard.me.

He's a painter and has launched SliceWalking, a project that support more than 40 families in 3rd world countries through art.slicewalking.com

He's 33 and wants to change the World!

General Information

Best Oral Presentation and Best Poster Presentation

All registered participants can vote for the awards during the period of the meeting. After voting the best oral and poster presentation (only one each), each participant should introduce their vote on the ballot box.

The prizes are a **FNAC voucher of 100€** and a **FNAC voucher of 50€** for bests Oral and Poster presentations, respectively, sponsored by **STAB VIDA**.

The award ceremony will be held at the closing dinner.

Meeting Closing Dinner

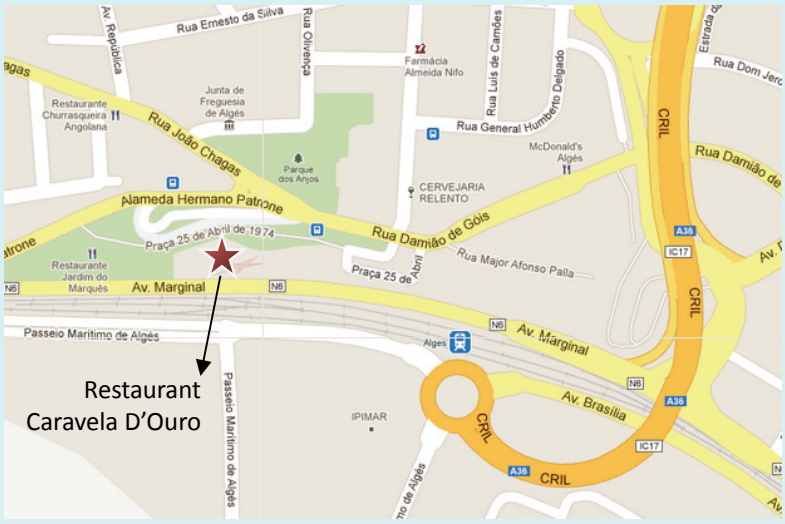
The meeting closing dinner is sponsored by Câmara Municipal de Oeiras.

The dinner will be held at the restaurant Caravela D'Ouro, Algés by 8p.m.

The restaurant is located near the train station of Algés.

Adress: Alameda Hermano Patrone, 1495-064 Algés

Tel. (351) 214 118 350



Note: please do not forget to bring your identification badge to the dinner.

Program

Day 1 (Wednesday, October 10)

09:00-10:00	**Registration**
10:00-10:15	**Opening – Luís Paulo Rebelo, Director of ITQB**
	Biology Session I Chairs: James Yates and Lia Domingues
10:20-10:40	**OP1. Renata Matos** *Enterococcus faecalis prophages are intricately interrelated and are important for adhesion to human platelets*
10:40-11:00	**OP2. Ana Tavares** *High prevalence of methicillin-resistant Staphylococcus aureus (MRSA) of hospital origin in the community in Portugal*
11:00-11:30	**Coffee-Break** – sponsored by NZYTech and Sociedade Portuguesa de Fisiologia Vegetal
	Biology Session II Chairs: Tiago Pais and Joana Rolo
11:30-11:50	**OP3. Ana Reis** *Functional characterization of Rrp44, the crucial component of the exosome*
11:50-12:10	**OP4. Inês Silva** *A Salmonella Typhimurium sRNA that controls the expression of a protein folding chaperone*
12:10-12:30	**OP5. Vânia Pobre** *High throughput technologies and detailed analysis reveal the importance of exoribonucleases and RNA sequence and structure in the control gene expression.*
12:30-13:30	**Lunch**
13:30-15:30	**Poster Session I: Biology and Plant Sciences** (Posters 1-9)
15:30-16:00	**Coffee-Break -** sponsored by Serdial
16:00-17:00	**Keynote Lecture** *"Genetic Conflicts"* Francisco Dionísio, FCUL
	Plant Sciences Session Chairs: Isabel Abreu and Liliana Ferreira
17:00-17:20	**OP6. Cátia Nunes** *Trehalose 6-phosphate primes SnRK1-regulated gene expression for growth in relation to sucrose availability*
17:20-17:40	**OP7. Nuno Almeida** *Differential expression of Lathyrus cicera and Lathyrus sativus transcriptomes in response to rust (Uromyces pisi) infection*
17:40	**Welcome reception** – sponsored by ITQB and IBET

Day 2 (Thursday, October 11)

	Technology Session I Chairs: Marta Justino and Filipe Almeida
9:40-10:00	**OP8. Joana Lamego** *A journey through the production and analysis of a human metabolising enzyme to the modification of an in vitro cell model*
10:00-10:20	**OP9. Ana Carina Silva** *Improving Peste des Petits Ruminants Vaccine production and stability*
10:20-10:50	**Coffee-Break –** sponsored by Omnilabor and Ordem dos Biólogos
10:50-11:50	**Keynote Lecture** *"Chromatin dynamics in African Trypanosoma"* Luísa Figueiredo, IMM
	Technology Session II Chairs: Marco Patrone and Pedro Silva
11:50-12:10	**OP10. Ana Filipa Rodrigues** *Using functional genomics for improved manufacture of virus-based biopharmaceuticals: Transcriptional profiling and metabolic engineering of retroviral vector producing cell lines*
12:10-12:30	**OP11. Cláudia Queiroga** *Disclosing Carbon Monoxide Protection in Cerebral Ischemia: Insights into the cellular mechanisms*
12:30-13:30	**Lunch**
13:30-15:30	**Poster Session II: Technology** (Posters 10-17)
15:30-16:00	**Coffee-break -** sponsored by Serdial
16:00-17:00	**Keynote Lecture** *"Bacterial proteins, a new class of multi-functional anti-cancer drugs"* Arsenio Fialho, IST
	Technology Session III Chairs: Pedro Mateus and Sowmiah Subbiah
17:00-17:20	**OP12. Isabel Martins** *Analysis of Aspergillus nidulans during biodegradation of recalcitrant substrates and ionic liquid stress*
17:20-17:40	**OP13. Rui Ferreira** *There's more to cork than sealing wine: plastics and drugs*
17:40-18:00	**OP14. Helga Garcia** *Suberin, making new from the old*

Day 3 (Friday, October 12)

	Technology Session IV Chairs: Bárbara Henriques and Neuza Teixeira
10:20-10:40	**OP15. Bárbara Almeida** *Micropollutants biodegradation in water and wastewater treatment systems: characterisation of the microbial populations involved in the removal process*
10:40-11:00	**OP16. Sandra Sanches** *Integration of membrane filtration and photolysis processes for drinking water treatment*
11:00-11:30	**Coffee-Break –** sponsored by ITQB
	Biological Chemistry & Chemistry Session I Chairs: Sofia Venceslau and Débora Tavares
11:30-11:50	**OP17. Eva Lourenço** *Synthesis of new protein stabilisers inspired by compatible solutes of hyperthermophilic microorganisms*
11:50-12:10	**OP18. Przemyslaw Nogly** *X-ray structure of a substrate-specific α-phosphoglucomutase from Lactococcus lactis at 1.5 Å resolution - a new member of the haloacid dehalogenase superfamily*
12:10-12:30	**OP19. Fábio Silva** *Hydrogen Metabolism in Desulfovibrio gigas*
12:30-13:30	**Lunch**
13:30-15:30	**Poster Session III: Biological Chemistry** (Posters 18-22)
	Biological Chemistry Session II Chairs: Catarina Pimentel and Carla Baltazar
15:30-15:50	**OP20. Maria Alves** *Molecular mechanisms of cytochromes involved in extracellular electron transfer*
15:50-16:10	**OP21. Mafalda Figueiredo** *Bacterioferritin protects the anaerobe D. vulgaris against oxidative stress conditions*
16:10-16:30	**OP22. Zélia Gouveia** *Targeting heme with single domain antibodies*
16:30-16:50	**OP23. Pedro Sousa** *Supercomplexes of Prokaryotic Aerobic Respiratory Chains*
16:50-17:20	**Coffee-Break –** sponsored by Serdial
17:20-18:50	**Interactive Session** **Miguel Gonçalves, SparkAgency**
20:00	**Closing Dinner, sponsored by Câmara Municipal de Oeiras** Award presentation ceremony for Best Oral and Poster Presentation, sponsored by STABVIDA

Posters

Poster Session I: Biology and Plant Sciences

PP1 Carriage of pneumococcal serotype 19A reduces *Staphylococcus aureus* carriage in Portuguese children in the era of PCV7
Débora A. Tavares, Ana Cristina Paulo, Hermínia de Lencastre, and Raquel Sá-Leão

PP2 Polymorphisms in Inc proteins and differential expression of *inc* genes among *Chlamydia trachomatis* strains correlate with invasiveness and tropism of lymphogranuloma venereum isolates
Filipe Almeida, Vítor Borges, Rita Ferreira, Maria José Borrego, João Paulo Gomes, and Luís Jaime Mota

PP3 Screen for novel type III secretion effectors of *Chlamydia trachomatis*
Maria Cunha and Luís Jaime Mota

PP4 Nitrogen-fixing biofertilizers for gramineous crops
Nádia Castanheira, Ana C. Dourado, Paula I. L. Alves, Alicia Cortés Pallero, M. Teresa Barreto Crespo, Nuno Borges, Paula Fareleira

PP5 Zeb1 function in neural stem/progenitor cells – a genomic approach
Pedro Rosmaninho, Vera Teixeira, Alexandre Raposo, and Diogo S. Castro

PP6 A reverse genetics approach to identify cardoon (*Cynara cardunculus*) genotypes with improved polyphenolic content
Ana Margarida Ferro, Rafael Lozano, Sónia Gonçalves, and Maria Margarida Oliveira

PP7 Understanding salt tolerance in plants: Two different approaches
Inês S. Pires, Sónia Negrão, Melissa M. Pentony, Isabel A. Abreu, Margarida M. Oliveira, and Michael D Purugganan

PP8 Chickpea seed protein digestibility and antinutritional content
Isa C. Ribeiro, Nuno Simões, Sébastien Planchon, Isabel Duarte, João B. Freire, Manuela Chaves, Jenny Renaut, and Carla Pinheiro

PP9 Differential expression of *Lathyrus cicera* and *Lathyrus sativus* transcriptomes in response to rust (*Uromyces pisi*) infection
Nuno Felipe Almeida, Susana Trindade Leitão, Björn Rotter, Peter Winter, Diego Rubiales, and Maria Carlota Vaz Patto

Poster Session II: Technology

PP10 Using functional genomics for improved manufacture of virus-based biopharmaceuticals: Transcriptional profiling and metabolic engineering of retroviral vector producing cell lines
Ana F. Rodrigues, Miguel Guerreiro, Ana S. Oliveira, Paula M. Alves, Wei-Shou Hu, and Ana S. Coroadinha

PP11 "Bioactivator" ionic liquids?
Diana Ruivo, Francisco J. Deive, Ana Rodriguez, José M. S. S. Esperança, and Luís P.N. Rebelo

PP12 Developing even higher ionicity ionic liquids (hiils). Simple: just add salt
F. S. Oliveira, A. B. Pereiro, J. M. M. Araújo, J. N. C. Lopes, Gabriel Feio, I. M. Marrucho, and L. P. N. Rebelo

PP13 Systems-level analysis of baculovirus-host interactions: from genomic to metabolomic decomposition
Francisca Monteiro, Vicente Bernal, and Paula M. Alves

PP14 New CO_2 separation membranes based on pyrrolidinium ionic materials
Liliana C. Tomé, David Mecerreyes, Carmen S.R. Freire, Luís Paulo N. Rebelo, and Isabel M. Marrucho

PP15 Awakening of silent secondary metabolites genes of two model Ascomycota by ionic liquids
Paula C. Alves, Diego O. Hartmann, Isabel Martins, Helga Garcia, Maria Cristina Leitão, Jörg Becker, and Cristina Silva Pereira

PP16 Production of helper-dependent canine adenovirus type 2 (CAV-2) vectors for gene therapy: Impact of transcomplementing gene products on producer cell-line
P. Fernandes, E.J. Kremer, A.S. Coroadinha, and P.M. Alves

PP17 New ionic liquids from renewable resources
S Sowmiah, C.A.M Afonso, J.M.S.S Esperança, and L.P.N Rebelo

Poster Session III: Biological Chemistry

PP18 Crz1 and Ca^{2+} - signaling in *Saccharomyces cerevisiae* exposed to arsenic stress
Rita T. Ferreira, Ana R. Courelas Silva, Catarina Pimentel, Liliana Batista-Nascimento, Claudina Rodrigues-Pousada, and Regina A. Menezes

PP19 Structural Virology of The Murid Gamma-Herpesvirus 4
Bruno Correia, Colin McVey, Marta Miranda, and Maria Arménia Carrondo

PP20 Unveiling specificities of the substrate pathways in a high activity H_2 producing enzyme
Carla S. A. Baltazar, Vitor H. Teixeira, and Cláudio M. Soares

PP21 Structural investigation of the NO sensing domain of the human soluble guanylate cyclase
Meire C. Almeida and Manolis Matzapetakis

PP22 Study of energy transduction in bacteriorhodopsin using continuum electrostatics methods
Pedro R. Magalhães, Sara R. R. Campos, and António M. Baptista

Oral Presentations

OP1. *Enterococcus faecalis* prophages are intricately interrelated and are important for adhesion to human platelets

Renata C. Matos[a,b,c], Nicolas Lapaque[b,c], Thierry Meylheuc[b,c], Lionel Rigottier-Gois[b,c], Francis Repoila[b,c], Bruno Gonzalez-Zorn[d], Maria de Fatima Lopes[a,e] and Pascale Serror[b,c]

[a]ITQB, UNL, Apartado 127, 2781-901 Oeiras, Portugal, [b]INRA, UMR1319 Micalis, F-78350 Jouy-en-Josas, France, [c]AgroParisTech, UMR Micalis, F78350 Jouy-en-Josas, France, [d]Universidad Complutense de Madrid, Spain, [e]IBET,Apartado 12, 2781-901 Oeiras, Portugal

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Polylysogeny is frequently considered as the result of an adaptive evolution process in which prophages confer fitness and/or virulence factors; making them important for evolution of both bacterial populations and infectious diseases. The *Enterococcus faecalis* V583 isolate belongs to the high-risk clonal complex 2 that is particularly well adapted to hospital environment. Its genome carries 7 prophage-like elements, one of which is ubiquitous in the species.

In this study, we addressed the activity of the V583 prophages and their role in bacterial virulence. We systematically analyzed the ability of each prophage to excise from the bacterial chromosome, to replicate and to package its DNA. We also created an array of *E. faecalis* strains that lack from single to all six non-ubiquitous prophages by mimicking natural excision.

Our work reveals, for the first time, that prophages of *E. faecalis* V583 can excise from the host chromosome in the presence of flourquinolones and are able to produce active phage progeny. Intricate interactions between V583 prophages were also revealed: prophages inhibiting excision of another prophage; prophage hijacking capsides from a helper prophage. Furthermore, prophages encoding platelet-binding like proteins were found to be involved in adhesion to human platelets, considered as a first step towards the development of infective endocarditis.

Our findings reveal not only a role for *E. faecalis* V583 prophages in virulence, but also supply an explanation for the correlation between antibiotic usage and *E. faecalis* success as a nosocomial pathogen, as the use of SOS-inducing antibiotics may promote gene exchange.

Keywords: *Enterococcus faecalis*, prophages, crosstalk, platelets, and adhesion.

OP2. High prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) of hospital origin in the community in Portugal

Ana Tavares[a], Maria Miragaia[a], Joana Rolo[a], Céline Coelho[a], and Hermínia de Lencastre[a,b] and Working group on CA-MRSA/MSSA*
[a]Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica (ITQB), Oeiras, Portugal, [b]Laboratory of Microbiology, The Rockefeller University, New York, NY, USA
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Methicillin-resistant *Staphylococcus aureus* were exclusively confined to hospitals (HA-MRSA) for several decades. However during the past 15 years they started to appear in the community (CA-MRSA) as well. CA-MRSA present specific genetic backgrounds and an enhanced virulence potential (e.g. Panton-Valentine leukocidin) [1,2]. In spite of their relevance in many countries, no data existed on the prevalence and epidemiology CA-MRSA in Portugal. In order to describe the *S. aureus* population structure and to assess its public health impact, a national survey was conducted.
Five hundred and twenty eight isolates were collected from individuals with no risk factors (for HA-MRSA acquisition) and were analyzed by molecular methods: pulsed-field gel electrophoresis, presence of *mecA* and PVL (*lukS-PV* and *lukF-PV*), SCC*mec* typing and SCC*mec*IV subtyping, multilocus-sequence typing and *spa* typing [3-7]. Antimicrobial susceptibility testing for a panel of 13 antimicrobial agents was performed Isolates relatedness was analyzed by the combination of the various typing techniques and by the Based Upon Repeat Patterns algorithm.
We found a relatively low prevalence of CA-MRSA (2.6%, 14/528) causing infection in Portugal that belonged to typical CA-MRSA epidemic clones (USA300, USA400, USA700, SWP, European and ST398) or less disseminated clones (ST121-IV_{NT}, ST1810-IVa).
Surprisingly, a relatively high prevalence of HA-MRSA (19.1%, 101/528) was found among the 528 isolates recovered; they were classified as belonging to the epidemic EMRSA-15, New York/Japan, EMRSA-16, Brazilian, Pediatric and Berlin clones. PVL was present in 3.2% of isolates (17/528) only. We found a high relatedness between the CA-MRSA and the CA-MSSA isolates, which indicates a local emergence of CA-MRSA.
The finding of CA-MRSA isolates that were multidrug resistant and isolates belonging to HA-MRSA epidemic clones suggests that CA-MRSA already entered into the hospital and that HA-MRSA may have gained a foothold into the community in Portugal. This finding is worrisome and of major public health concern.

References:
[1] Watkins R.R., David M.Z., Salata R.A., *J Med Microbiol* , 2012, 61, 1179; [2] David M.Z. and Daum R.S., *Clin Microbiol*, 2010,23, 616; [3] Lina G., Piemont Y., Godail-Gamot F., Bes M., Peter M.O., *Clin Infect Dis*, 1999, 29, 1128; [4] Milheirico C., Oliveira D.C., de Lencastre H., *Antimicrob Agents Chemother*, 2007, 51, 3374; [5] Milheirico C., Oliveira D.C., de Lencastre H., *J Antimicrob Chemother*, 2007, 60, 42 ; [6] Enright M.C., Day N.P., Davies C.E., Peacock S.J., Spratt B.G., *J Clin Microbiol*, 2000, 38, 1008; [7] Shopsin B., Gomez M., Montgomery S.O., Smith D.H., Waddington M., *J Clin Microbiol*, 1999, 37, 3556

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), community-associated (CA)-MRSA, hospital-associated (HA)-MRSA, epidemiology

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OP3. Functional characterization of Rrp44, the crucial component of the exosome

Filipa Pereira Reis[a], Ana Barbas[a], Daneen Schaeffer[b], A Alejandra Klauer[b], Ambro van Hoof[b] & Cecília Maria Arraiano[a]
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RNA maturation, turnover and quality control are essential for cell viability. In eukaryotes, the exosome plays a central role in all these processes. The core exosome is composed of nine catalytically inactive subunits constituting a ring plus an active exoribonuclease that hydrolyzes RNA to mononucleotides acting in the 3´-5´ direction. The nuclease Rrp44, also known as Dis3, is the enzyme responsible for the activity of the exosome. All core subunits are highly conserved between eukaryotes and are essential for viability.
We showed for the first time that a Rrp44/Dis3 protein that lacked the RNB catalytic domain still had nuclease activity [1]. However, this activity was lost when the N-terminal PIN domain was mutated. This proved that in addition to exonucleolytic activity, Rrp44 also has endoribonucleolytic activity, associated with its PIN domain. These results brought important implications on the mode of action of the exosome, namely the mechanism of recruitment and degradation of RNA, and the model of the exosome had to be revisited.
Rrp44 is a member of RNase II superfamily of exoribonucleases. The structure of RNase II allowed the explanation of all the known properties of the enzyme and elucidated the dynamic mechanism of RNA degradation [2]. When Glu542 was changed to Ala*, E. coli* RNase II became a "Super-Enzyme" with 120 fold increase in activity [3]. We studied the role of the corresponding Gln892 residue in yeast Rrp44. When we replaced the Gln892 residue with Ala the enzyme became slightly more active *in vitro*. We have mutagenized other aminoacids and we were able to change the size of the end-product with accumulation of degradation products and consequences for cellular growth. These findings are quite important since the determination of the role of the conserved residues in Rrp44 is essential to understand how the exosome ensures proper cellular RNA metabolism.

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Keywords: Exosome, Ribonuclease, RNA degradation, Rrp44, *S. cerevisiae*

OP4. A *Salmonella* Typhimurium sRNA that controls the expression of a protein folding chaperone

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Small non-coding RNAs (sRNAs) perform a wide diversity of regulatory functions in both prokaryotic and eukaryotic cells. These regulatory molecules are generally highly expressed in the cell to face a variety of different stresses, including entry in stationary phase of growth. SraL sRNA is an example of a sRNA highly expressed in stationary phase. This sRNA was firstly described in *Escherichia coli* and was recently characterized in *Salmonella enterica* serovar Typhimurium [1]. Its expression is also high under SPI-2 inducing conditions.

In this work we show that SraL is directly transcribed by the major stress sigma factor σ^S (RpoS) in *Salmonella*. We also demonstrate that SraL sRNA down-regulates the expression of the chaperone Trigger Factor (TF), encoded by the *tig* gene. TF assists protein folding by protecting the nascent peptide chains during synthesis and initial folding stages and by accelerating the peptidyl-prolyl *cis*/*trans* isomerization. By using bioinformatic tools and mutagenesis experiments, SraL was shown to interact with the 5'-UTR of the tig mRNA few nucleotides upstream of the Shine-Dalgarno region. This work constitutes the first report of a small RNA affecting protein folding. Taking into account that both SraL and TF are very well conserved in enterobacteria this work will have important repercussions in the field.

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Keywords: trigger factor, Sigma S (RpoS), sRNA, protein folding, SraL

OP5. High throughput technologies and detailed analysis reveal the importance of exoribonucleases and RNA sequence and structure in the control gene expression.

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Bacterial small regulatory RNAs (sRNAs) parallel microRNAs in their ability to control multiple targets. sRNAs usually act by an antisense mechanism and bind their target mRNAs inhibiting translation and promoting decay. Therefore it is important to study the degradation of sRNAs and how they promote the decay of their targets. We have characterized RNA determinants involved in sRNA stability and analyzed how they influence the expression of its targets. Our study model was the sRNA MicA that represses several genes, including major outer membrane proteins like ompA, lamB and ecnB. Site-directed mutagenesis was used to construct MicA mutated forms. The 5'linear domain, the structured region with two stem-loops, the A/U-rich sequence or the 3' poly(U) tail were altered without affecting the overall secondary structure. The stability and the target regulation abilities of the wild-type and the different MicA mutants were compared. Our results show that the diverse MicA modules differently affect MicA stability and target control.

Ribonucleases are the effectors that recognize and degrade not only small RNAs like MicA but also all the mRNAs in the cell. Therefore they can have a major impact in the control of gene expression. In *Escherichia coli* there are three 3'-5'exoribonucleases that accomplish most of the RNA degradative activity: RNase II, RNase R and PNPase. In this work we used next-generation sequencing to study the roles of the different exoribonucleases in the cell. We did whole genome sequencing of the wild-type and the mutants for the different exoribonucleases grown in different conditions. This approach allowed us to have a broader image of the role of these exoribonucleases in the cell.

In conclusion, this work expanded our knowledge on how RNA degradation is carried out in the cell and opened new perspectives for the engineering of non-coding RNAs that selectively choose among multiple targets.

Keywords: RNA degradation, Small non-coding RNAs, Exoribonucleases, MicA, RNA modules

OP6. Trehalose 6-phosphate primes SnRK1-regulated gene expression for growth in relation to sucrose availability

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Trehalose 6-phosphate (T6P) is a plant sugar signal that inhibits the central regulatory SNF1-related protein kinase, SnRK1. T6P responds to sugar availability and inhibits SnRK1 which alters gene expression and promotes growth. This provides a model for the regulation of growth by sugar availability. However, it is not known how this model operates under sink-limited conditions when tissue sugar content is uncoupled from growth. To test this, Arabidopsis seedlings were transferred to low temperature or zero nitrogen over a 72-h period and contrasted to sucrose and glucose feeding under optimal conditions. Maximum *in vitro* activities of SnRK1 changed little, but in contrast, large increases in T6P, up to 55-fold, were proportional to tissue sucrose content in all treatments. SnRK1-induced and SnRK1-repressed marker gene expression showed a strong relationship with T6P above and below a threshold level of 0.5 nmol.g^{-1} FW calculated as close to the dissociation constant (5 µM) of the T6P/SnRK1 complex. This occurred irrespective of the growth response to sucrose. Once growth-limiting conditions were relieved, however, such as upon transfer from cold to warm, T6P enabled rapid growth. Glucose feeding did not increase T6P content and changes in SnRK1 marker genes were more moderate, which may result from less potent inhibition of SnRK1 by glucose 6-phosphate and glucose 1-phosphate. It is concluded that T6P is primarily a signal of sucrose availability which controls SnRK1-regulated gene expression, priming growth to proceed when sucrose is above starvation levels once other constraints, such as low temperature, are removed.

Keywords: Sugar, trehalose 6-phosphate, SnRK1, gene expression, growth, *Arabidopsis thaliana*

OP7. Differential expression of *Lathyrus cicera* and *Lathyrus sativus* transcriptomes in response to rust (*Uromyces pisi*) infection

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Chickling pea (*Lathyrus cicera* L.) and grass pea (*Lathyrus sativus* L.) have great potential among grain legumes due to their good adaptability to inauspicious environments, high protein content and resistance to serious diseases. Due to the need of increasing sustainability of agricultural production systems the interest in the conservation and utilization of *Lathyrus* genetic resources is rising. Nevertheless, due to its past underuse, just a few molecular breeding tools were developed specifically for *Lathyrus* species. Research on more important economically related legumes can to a certain extend contribute to surpass this limitation.
We have compared the leaf transcriptome profiles of two contrasting accessions each of *L. cicera* and *L. sativus* in rust (*Uromyces pisi*) infected and non infected conditions. For *L. cicera* a normalized cDNA library was created using Illumina sequencing. For *L. sativus* a semi-quantitative RNA-seq method was chosen in order to detect also variation in expression level.
These new data will allow bridging *Lathyrus* genomic information with model species and better studied related legume crops in order to develop molecular tools that will allow breeding these ancient crops interesting for modern farmers.
Results will be discussed.

Keywords: Lathyrus, Gene Expression Profiling, *Uromyces pisi*, Disease resistance

OP8. A journey through the production and analysis of a human metabolising enzyme to the modification of an *in vitro* cell model

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This presentation will be focused on Carboxylesterases (CESs), which are phase I metabolising enzymes involved in the processing of well-known therapeutic agents such as aspirin, cocaine and heroin. Carboxylesterase 2 (hCES2) is the main CES expressed in human intestine, despite being down-regulated in the most used *in vitro* intestinal cell model for permeability, the human colon adenocarcinoma derived Caco-2 cell line.

The manufacturing of recombinant hCES2 in human embryonic kidney cells (HEK-293T),[1] and its characterization through a newly developed methodology,[2,3] allowed to unravel new fundamental properties such as the presence of oligomeric active and inactive forms of hCES2. Although other human CESs, such as carboxylesterases 1 (hCES1), were shown to exist as trimers and hexamers, hCES2 had been previously reported to exist only as a 60 kDA monomer. These new findings suggest the oligomerisation may be a common feature for CESs, challenging the current knowledge about these enzymes.

Fitted with a deep knowledge about hCES2, this enzyme was overexpressed in Caco-2 cells[4]. This cell line, mimicking human enterocytes, fails in the accurate absorption prediction of ester-containing drugs and prodrugs, especially of those metabolised by hCES2. A hCES2-expressing Caco-2 cell line was generated through clonal selection, having the potential of becoming a powerful tool for the drug development industry.

Through this scientific journey from a human metabolising enzyme to a human intestinal cell model, an example on how fundamental and applied research may work together to improve current knowledge and manufacturing practices will thus be provided.

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Keywords: carboxylesterase 2, Caco-2 cells, human protein manufacturing, cell line development, analytical techniques development.

OP9. Improving Peste des Petits Ruminants Vaccine production and stability

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Peste des Petits Ruminants (PPR) is an highly contagious and fatal disease of sheep and goats and is considered as one of the major constraints to the production of small ruminants in Africa and Asian countries. For the control of PPR, an attenuated vaccine is available (PPRV Nigeria 75/1) which is produced in monolayer cultures of Vero cells. PPR virus is heat sensitive which makes challenging an efficient use of the vaccine in the endemic areas that have hot climatic environments. This work aims at developing a scalable production process for a PPR vaccine and at improving the final formulation of the vaccine to increase stability. The results to be presented provide insights into the feasibility of applying microcarrier cell culture technology to produce PPR vaccine in Vero cells. As alternative, cell lines adapted to grow as single cells in suspension (CHO-K1, BHK-21A and 293) using serum free media were also evaluated in their capacity to produce the PPR vaccine. BHK-21A and 293 cells were both suited to produce PPR vaccine with productivities similar to Vero cells, namely 10^6 $TCID_{50}$/ml. To improve the stability of the current PPR vaccine, new formulations based on the Tris buffer were tested and compared with the formulation currently used, the Weybridge medium. The results showed that it is possible for the PPR vaccine to have adequate short-term stability at non-freezing temperatures to support manufacturing, short-term shipping and storage using a Tris/Trehalose formulation. The new candidate formulation derived from this work has been already transferred to one of the manufacturing laboratories of the PPR vaccine in Africa, the National Veterinary Institute (NVI) in Ethiopia. This more stable formulation should significantly enhance the potential of the vaccine in the control of a PPR outbreak.

Keywords: Peste des Petits Ruminants Vaccine, Serum Free Medium, Scalable production process, Stability, Formulation, Storage.

OP10. Using functional genomics for improved manufacture of virus-based biopharmaceuticals: Transcriptional profiling and metabolic engineering of retroviral vector producing cell lines

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Recombinant retroviruses comprise several valuable biopharmaceuticals such as gene therapy vectors, recombinant vaccines and replicative-competent particles for oncolytic therapy. However, their manufacture faces challenges in several fronts, particularly the low-yields, high non-functional particles content and the animal sera required in to sustain long-term productivity. Our limited knowledge on the biochemical pathways related to virus production has impeded cellular and process engineering efforts to enhance production performances. In this PhD project they were examined the transcriptome and metabolome profiles in two scenarios: a retrovirus producer cell establishment and virus production under serum deprivation. A systems approach was taken to integrate the data and gain new insights on the process, particularly, to find gene targets for metabolic engineering for improved cell productivities.
During a producer cell establishment 12 metabolic networks were found to be recruited and more than 30 genes identified as potential candidates for metabolic engineering. Media manipulation targeting these pathways resulted in productivity improvements from 1.3 to 2.1 fold when targeted individually and, when in combination, a synergistic effect resulting in a 6 fold increase. Two of these pathways were chosen for gene engineering, resulting in more than 20-fold improvement of vector productivity.
During a serum deprivation challenge, lipids, particularly cholesterol, were identified as the main component necessary for virus production. The characterization of cell lipid content using ^{13}C-NMR spectroscopy revealed differences in lipid biosynthetic capacity responsible for serum heterotrophy. These differences were scrutinized using transcriptional profiling and two key players were identified: AMPK and the down-regulation of lanosterol synthase, an enzyme of cholesterol biosynthesis machinery.
This project is currently proceeding through the development of a high-throughput screening method enabling a large-scale metabolic engineering study to test all the potential gene targets identified and their combination, in the pursuit of in-depth knowledge of the metabolic hotspots of viral replication and improved producing phenotypes.

Key words: retroviral-based biopharmaceuticals; functional genomics; systems biology; metabolism; lipids

OP11.Disclosing Carbon Monoxide Protection in Cerebral Ischemia: Insights into the cellular mechanisms

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Cerebral preconditioning (PC) is defined as an insult below damage threshold that induces a tolerance state in the organism. PC induction can be a therapeutic strategy to limit cerebral ischemia lesion by activating endogenous protective mechanisms. Indeed, clinical trials are under development [1]. Carbon monoxide (CO) is an endogenous molecule commonly known for being toxic due to its high affinity to heme [2]. However, low doses of CO play a beneficial role in several systems [3-5]. Previously we have shown that CO prevents neuronal cell death [6]. The aim of this thesis work is to disclose the mechanisms through which CO induces PC in order to prevent cell death. In primary cultures of astrocytes, CO treatment inhibits apoptosis. Moreover, in brain isolated non-synaptic mitochondria, CO induces a post-translational modification (glutathionylation) in adenine nucleotide translocase (ANT) avoiding its lethal conformation and mitochondrial membrane permeabilization (MMP), being a critical event in the apoptotic process [7]. Also, CO has the ability to reinforce oxidative phosphorylation by augmenting specific cytochrome c oxidase (COX) activity and mitochondrial biogenesis [8]. Furthermore, Bcl-2 interaction with COX is an important step for CO-induced protective effects. Using a model of primary co-cultures of neurons and astrocytes (2D), it was shown that neurons cocultured with CO-treated astrocytes are more resistant against cell death. Purinergic molecules are involved in this CO non-cell autonomous role. Also, in a rat perinatal model of brain hypoxia-ischemia (HI), Vannucci model, CO exposition clearly decreases apoptotic cell death in the hippocampus [9]. Using histological, immunohistochemical and immunoblotting tools, it was shown that CO treatement increases anti-apoptotic machinery and lowers pro-apoptotic events. Therefore, CO might be faced as a promising and novel therapeutic agent against brain HI injury, by upregulating neuroprotective cellular mechanisms, via PC induction.

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Keywords: Carbon monoxide, preconditioning, brain, mitochondria, apoptosis

OP12. Analysis of *Aspergillus nidulans* during biodegradation of recalcitrant substrates and ionic liquid stress

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Ionic liquids (*i.e.* organic salts with a melting temperature below 100 °C) generally exhibit astonishing properties, namely outstanding solvent quality and negligible vapour pressure. The last property has rendered ionic liquids an early classification as "green" solvents. However, ionic liquids are a very disparate class of chemicals and any generalization concerning their properties should be avoided[1]. Many are in fact toxic and environmental persistent.

Making use of these chemicals, the Applied and Environmental Mycology team (AEM), together with members of the Molecular Thermodynamics laboratory, have efficiently and specifically attained suberin extraction from cork[2]. Though this plant polyester is highly resistant to biodegradation; filamentous fungi are amongst the seldom described organisms able to degrade it [3]. In addition, these organisms have also been shown to tolerate high concentrations of ionic liquids [4].

The biotechnological exploitation of fungal bio-transformation of suberin (after its extraction form cork) is being aimed at. Therefore, fungal biotransformation of suberin and fungal response during exposure to ionic liquids need to be investigated. The molecular basis of these processes can be better understood using an "omics" approach.

In this work, a proteomic "snap-shot" view of the major alterations induced by ionic liquids in *Aspergillus nidulans* was done. The obtained data showed that major alterations were related to the carbohydrate metabolism and stress-response. Amongst the differentially expressed proteins we identified some which are characteristic of different developmental stages, suggesting that the ionic liquids exposure had profound repercussions on the fungal developmental cycle. Using both proteomics and transcriptomics we are currently analyzing the fungal suberin degradation processes. Our goal is to identify putative genes/proteins involved in the suberin degradation; especially those which responds to ionic liquid stimuli.

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Keywords: filamentous fungi, proteomics, transcriptomics, ionic liquids, suberin

OP13. There's more to cork than sealing wine: plastics and drugs

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During the last decade, we have been witnessing an exponential increase of interest in the extraction of valuable chemicals, materials and fuels from renewable resources, namely by-products of agro-forest industries.
Amongst natural resources, cork is one of the most remarkable materials. Its chemical and structural complexity hides numerous added-value compounds, from macro-monomers to triterpenes [1]. Currently, cork is essentially used to produce stoppers. This generates a high amount of residues (*ca.* 25 wt% of the total production), which can become profitable if adequately exploited. The most critical aspect is to develop sustainable isolation methods for cork added-value compounds. This may rely on the use of green solvents, such as functionalised ionic liquids (ILs) [2], or alternative extraction technologies, such as microwaves [2].
ILs are liquid salts, solely constituted by ions, which usually present high solvent ability. The plasticity of their synthesis allows a task-design approach, *i.e.* fine-tuning of their properties (*e.g.* polarity and H-bonding ability) to fulfil a particular process requirement.
A new process for suberin extraction has been completely developed by the authors at ITQB [3,4]. It has been demonstrated that cholinium hexanoate, a bio- compatible/degradable IL, can efficiently and selectively extract suberin domains from cork. It has been already shown that the IL-based process can also be applied to isolate suberin from birch bark [5] and potato peels (unpublished data). This IL-extracted suberin displays unique properties, very distinct from those of suberin obtained by conventional extraction processes. In addition, extraction of triterpenes, such as betulin, from cork and birch bark is currently under development. The obtained data shows that microwave assisted extraction is the most promising technology.
Our vision is to achieve the maximum waste valorisation, developing an integrated process that isolates sequentially tritrepenes and suberin.

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Keywords: suberin, ionic liquid, cholinium alkanoates, cork, birch bark, ATR-FTIR

OP14. Suberin, making new from the old

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Suberin, a complex aromatic-aliphatic cross-linked biopolyester, is widespread in the plant Kingdom but particularly abundant in *Quercus suber* L. cork (30-50 wt%) and *Betuta pendula* outer bark (40-50 wt %) [1,2]. This hydrophobic biopolyester plays a key role as a protective barrier between the plant and the environment. Suberin constitutes a major natural source of valuable compounds, such as ω-hydroxyacids, α,ω-dicarboxylic acids and corresponding mid-chain epoxy or dihydroxy derivatives [1,3]. These compounds have attracted considerable attention as building blocks for polymer synthesis [1].
Wastes derived from birch kraft pulp mills and cork industries are produced in large amounts, corresponding to *ca*. 3.4 wt % and *ca*. 23 wt % of the total production, respectively [3]. These wastes are generally burned to produce energy [3], though substantial valorisation could be attained if valuable components are extracted prior to burn.
Suberin can be isolated from cork and birch outer bark wastes by a set of defined depolymerisation methodologies, which normally require harsh alkaline processes. The successful green extraction of suberin from cork with cholinium hexanoate [4,5] prompted us to isolate also suberin from birch outer bark [6]. In the present study, a comparative analysis focussing on the chemical composition and the thermal behaviour of suberin samples was attained [6]. Suberin composing monomers were similar to those detected in samples obtained by conventional depolymerisation processes, however samples extracted by the ionic liquid present apparently unique features. The suberin samples extracted from cork and birch outer bark with cholinium hexanoate were observed to be mainly composed by oligomeric/polymeric fractions, still cross-linked, with a comparable thermal behaviour to that of the starting materials. A major breakthrough is the direct use of these samples as macromonomers for the development of novel biopolymeric materials, which are currently being characterised. This will also provide new opportunities for solving suberin *in situ* structure, still under debate.

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Keywords: suberin, biopolymer, cholinium hexanoate, *Quercus suber* L. cork, *Betuta pendula* outer bark

OP15. Micropollutants biodegradation in water and wastewater treatment systems: characterisation of the microbial populations involved in the removal process

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The contamination of freshwater with a variety of pollutants and the rapid decrease in the availability of quality water is a subject of concern worldwide [1]. Many of these compounds are toxic to public health and environment [1-2]. Wastewater treatment plants (WWTP) and *in situ* bioremediation are treatment measures of water contamination that use microbial degradation, with clear economical and environmental advantages [2]. Therefore, it is important to characterise such microbial populations, to identify which community members and groups of microorganisms have a role in micropollutants biodegradation and what process conditions can promote their selection in water treatment systems.

In this context, aspects impacting the biologically-mediated removal of two non-steroidal anti-inflammatory drugs (ibuprofen and ketoprofen) and a heavy metal (mercury) by microbial communities were investigated. The biodegradation of ibuprofen and ketoprofen by activated sludge has been found to follow different kinetics in various studies using different sludges, although the same profile was observed with a lag-phase occurring before degradation took place. A mathematical model was developed to describe this performance at different initial ibuprofen and ketoprofen concentrations. Regarding the heavy metal removal study, the choice of carbon source (glucose, acetate or ethanol) played a major role both in the microbial community structure and mercury reducing performance.

Several isolates were identified able to degrade ibuprofen, ketoprofen or mercury. Most notably, *Patulibacter medicamentivorans*, a new species, was discovered and characterised. This microorganism was found to biodegrade ibuprofen as a function of the biomass growth rate and ibuprofen concentration, which was incorporated into a mathematical model. Moreover, it was used in a proteogenomic approach to investigate the genes and proteins induced in the presence of ibuprofen. The results revealed the up-regulation of proteins related to up-take and degradation of aromatic acids.

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Keywords: water pollution, micropollutants, degradation, microbial communities, pure cultures

OP16. Integration of membrane filtration and photolysis processes for drinking water treatment

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The occurrence of micropollutants in drinking water sources has raised public attention due to their detection at ng/L and □g/L levels in several surveys [1]. The increasing evidence of the impact of emerging contaminants on aquatic environments and human health urges the development of new technologies that allow utilities to provide high quality water. Even though low pressure ultraviolet (UV) radiation and nanofiltration have proved to effectively remove several micropollutants from water, drawbacks related to their application have been identified: formation of potentially toxic by-products during UV photolysis as well as the formation of fouling during nanofiltration and the need to further treat/dispose the highly concentrated retentate [2]. The integration of these two processes was evaluated to assess its potential to remove several endocrine disrupting hormones as well as priority pesticides and polycyclic aromatic hydrocarbons (PAHs) – Directive 2000/60/EC – from natural water sources as well as to overcome the drawbacks associated with the treatment processes.

Advanced oxidation processes, using hydrogen peroxide and titanium dioxide, did not improve the degradation of the selected pesticides while it highly enhanced the degradation of some hormones. The water composition impacted the degradation of the PAHs (13-93%) and the production of photolysis by-products while it showed no significant effect on the high removal of the pesticides (often >60%). Nanofiltration was very effective removing the PAHs (>99%) while the removals of pesticides and hormones were often higher than 67%. Size exclusion as well as hydrophobic and electrostatic interactions were identified as the main rejection mechanisms during nanofiltration. The integration of the two technologies was effective removing the pesticides and hormones as well as decreasing the estrogenic activity of the treated water by retaining the target compounds and the formed by-products. The estrogenic activity of the retentate containing hormones highly decreased, reducing the need to conduct further treatment.

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Keywords: Water treatment, LP/UV photolysis, nanofiltration, pesticides, hormones, natural water matrices

OP17. Synthesis of new protein stabilisers inspired by compatible solutes of hyperthermophilic microorganisms

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In response to osmotic or heat stress, marine hyperthermophiles (thriving optimally above 80ºC) accumulate ionic compatible solutes such as α-D-mannosyl-D-glycerate (MG) [1]. It has been proposed that these compounds stabilise intracellular components, such as proteins and enzymes, allowing them to withstand high growth temperatures. Furthermore, the efficacy of these solutes in the stabilisation of a number of model proteins has been demonstrated in vitro. Since several human pathologies such as Alzheimer's, Creutzfeldt-Jacob's, cystic fibrosis and Parkinson's diseases have been associated with the structural instability of proteins, and consequent protein aggregation, the development of reliable strategies to improve protein stability is of great importance and could lead to several pharmaceutical and biotechnological applications.

Strategies for the synthesis of newly isolated natural solutes, α-D-mannopyranosyl-(1→2)-α-Dglucopyranosyl-(1→2)-D-glycerate **1** (MGG), (2R)-2-(1-O-α-D-mannopyranosyl)-3-(1-O-α-Dglucopyranosyl)-glycerate **2** and α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-Dglycerate **3** (GGG), were developed both in solution and using a solid support (Figure 1). Chemical synthesis provided a convenient method to obtain, study and confirm the structure of the isolated compounds which often are present in very limited quantities in their natural producers.

Several new synthetic analogues derived from different hexoses chemically related to MG have been synthesised and their thermostabilisation properties assessed. In order to enhance the anomeric selectivity of the glycosylation reaction for the preparation of new 1,2-cis galactose and glucose analogues, different thioglycoside activated donors were prepared. The influence of the nature of the protective groups on different positions of the donor, solvent and temperature on the stereochemical outcome of these glycosylation reactions was studied using a wide range of acceptors.

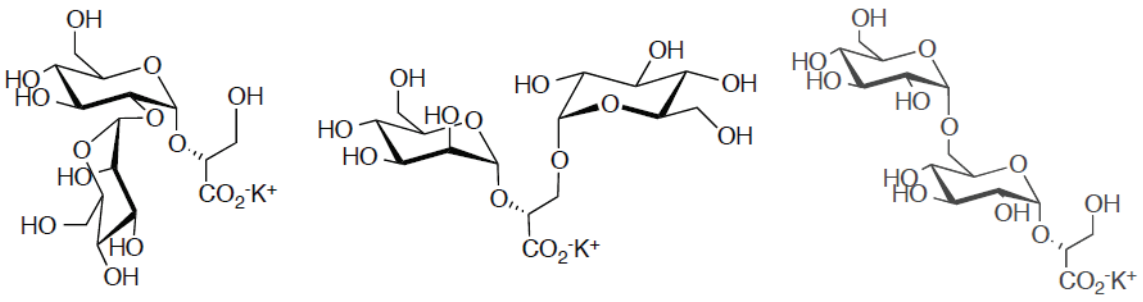

Figure 1.

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Keywords: hypersolutes, glycosylation reaction, solid supported synthesis.

OP18. X-ray structure of a substrate-specific α-phosphoglucomutase from *Lactococcus lactis* at 1.5 Å resolution - a new member of the haloacid dehalogenase superfamily

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α-Phosphoglucomutase (α-PGM) is an enzyme that is essential for the growth of *Lactococcus lactis*. The enzyme links bacterial anabolism with sugar utilization through glycolysis by catalyzing the reversible interconversion of glucose 6-phosphate and α-glucose 1-phosphate. The gene encoding α-PGM was cloned and overexpressed in *L. lactis*. The purified protein was functionally active and was crystallized using vapour-diffusion [1]. Crystals were optimized applying seeding techniques, and using experimental phasing the protein model was built and refined to high resolution of 1.5 angstroms.
The studied enzyme is not related to the common α-phosphoglucomutases within the α-D-phosphohexomutase superfamily. Instead it shows homology to the haloacid dehalogenase superfamily, with 24% sequence identity to the human phosphomannomutase 1. Unlike the related eukaryotic phosphomannomutases, the lactococcal enzyme shows strict specificity for α-glucose 1-phosphate and does not process α-mannose 1-phosphate [2]. This first structure reveals a novel dimeric assembly of the protein and provides insights into the strict substrate differentiation mechanism of the enzyme.

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Keywords: protein structure, phosphoglucomutase, substrate specificity, enzymatic mechanism

OP19. Hydrogen Metabolism in *Desulfovibrio gigas*

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Molecular hydrogen is an important component of diverse metabolic pathways, playing an active role in the metabolism of carbon compounds in bacteria. Several *Desulfovibrio spp*, *Desulfovibrio gigas* among them, are able to use hydrogen as sole energy source due to the presence of an enzymatic group called hydrogenases. In *D. gigas*, two hydrogenases are described so far: a periplasmic hydrogenase belonging to the [NiFe] group (*hynAB*) and a cytoplasmic membrane-bound hydrogenase (*ech*) previously described by our lab [1]. As such, the hydrogen cycling model, originally proposed by Odom and Peck [2] as a mechanism of energy conservation, could operate under lactate growth. Other models propose that hydrogenase activity could also be used simply as a way of regulating the cells redox state. To better understand the role of the hydrogenases in the cell metabolism and the contribution of each of these enzymes, two mutant strains lacking either the Ech or the HynAB hydrogenases were constructed, and their physiology was analyzed. Also, expression profiles of both *ech* and *hynAB* hydrogenases, at the level of mRNA and protein, are being evaluated. The data obtained so far suggest that the protein responsible for H_2 production in fermentative conditions (absence of sulfate) is the HynAB hydrogenase and not the Ech hydrogenase, since the Δ*ech* mutant shows an increased H_2 production compared to the wild type. This result is surprising since the Ech hydrogenase is expected to produce H_2. Nevertheless, when H_2 is the only energy source, HynAB clearly acts as a H_2 consuming hydrogenase since its deletion impairs growth. All together these results do not support the hydrogen cycling model as an essential mechanism in the conditions tested. The HynAB Hase can act reversibly, depending on the redox conditions, whereas the role of the Ech Hase is still unclear.

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Keywords: hydrogenase, metabolism, molecular hydrogen, mutant, *ech*, *hynAB*

OP20. Molecular mechanisms of cytochromes involved in extracellular electron transfer

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In recent years, new methods of clean and environmentally friendly energy production, such as Microbial Fuel Cells (MFCs), have been the focus of intense research efforts. The natural habitats of bacteria thriving in MFCs are usually marine and freshwater sediments. These bacteria typically have a remarkably versatile bioenergetic metabolism, often associated with formation of biofilms and the exchange of electrons with the surface of solids outside of the cell.
Several organisms have been the focus of research because of their capability to donate/accept electrons to/from an anode, such as *Geobacter* and *Shewanella*/*Rhodopseudomonas* and *Rhodobacter,* respectively.
The use of extracellular electron acceptors and donors requires a novel arrangement of the respiratory chains, to couple electron transfer to and from the outside of the cell to the production of ATP at the inner membrane. A hallmark of organisms performing extracellular electron transfer (EET) is the presence of many redox proteins including various cytochromes annotated in their genomes.
One of the most studied species that perform EET is *Shewanella oneidensis* MR-1, whose genome contains numerous c-type cytochromes. Several of these cytochromes have been implicated in the bioenergetic metabolism that uses soluble and insoluble metal compounds as terminal electron acceptors.
The detailed functional characterization of the small tetraheme cytochrome (STC or CctA) from *S. oneidensis* MR-1 and *S. frigidimarina* showed that there is functional specificity and directional electron transfer, despite the absence of specific interactions with physiological partners. This reveals that individual protein characteristics can be important in determining the electron flow direction.
This research aims to identify the amino acids that are important in the electron transfer mechanism between STC and metal oxides, and interactions with physiological redox partners. For this purpose, 15 mutants of surface residues were created and their detailed thermodynamic and kinetic characterization will be presented.

OP21. Bacterioferritin protects the anaerobe *D. vulgaris* against oxidative stress conditions

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Sulfate-reducing microorganisms which include *Desulfovibrio* species are commonly found in waterlogged soils containing abundant organic material where they contribute positively to bioremediation of several toxic metals. However, due to their metal corrosion ability, *Desulfovibrio* sps. have a strong negative economic impact constituting a major problem in oil and gas industries. These microorganisms grow under anaerobic conditions, but their presence in oxic-anoxic interfaces together with their need for iron implies that pathways for defense against toxic reactive oxygen species (ROS), generated via the Fenton reaction, are active in these bacteria.
In this work, we investigated the oxygen sustaining mechanisms active in *Desulfovibrio* sps. To this end, we have analyzed the effect of different oxygen concentrations on the expression of several genes putatively involved in the *Desulfovibrio vulgaris* oxygen resistance, using transcriptomic and physiological approaches. We observed that among the several genes studied the iron storage protein bacterioferrin (*bfr*) exhibited the highest induction in cells exposed to oxygen. Hence, the *D. vulgaris bfr* mutant strain was analyzed which revealed that in the absence of the gene the survival ability of *D. vulgaris* in oxygenated cultures significantly decrease. Furthermore, we found that the expression of *D. vulgaris bfr* is dependent on the general peroxide regulator PerR.
Altogether, these results reveal, for the first time, that bacterioferritin contributes to the oxygen resistance of the anaerobe *D. vulgaris.*

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Keywords: *Desulfovibrio*, iron, oxygen stress, bacterioferritin.

OP22. Targeting heme with single domain antibodies.

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Heme, i.e. iron (Fe) protoporphyrin IX, functions as a prosthetic group in a variety of hemoproteins essential to support life. Heme can catalyze the production of cytotoxic reactive oxygen species and presumably for this reason it must be sequestered within the heme pockets of hemoproteins, controlling its reactivity. Some pathologic conditions can be associated with heme release from hemoproteins, e.g. hemoglobin. While the released heme is not cytotoxic per se, in becomes cytotoxic in the presence of a range of inflammatory mediators (*1, 2*). This can promote tissue damage and exacerbate the severity of pathologic conditions such as malaria (3) caused by *Plasmodium* infection, or severe sepsis caused by polymicrobial infections (2). Here we describe and characterize a panel of single domain antibody fragments (SdAbs) targeting specifically heme that is not bound to the heme pockets of hemoproteins. We used a phage-display library with variability on the light-chain complementarity determining region (CDR)1 and CDR3 amino acids, replacing twelve codons with a mismatched random nucleotide sequence, screening the mutant library for recognition and functional specificity. The light-chain was derived from a single-chain antibody selected from a rabbit-derived combinatorial library (4). When separated, both heavy and light chains show high stability and expression after "camelization" of the heavy chain (5). The inherent stability of the light-chain allows variability and extension of CDR1 and CDR3, generating a synthetic antigen-binding library accommodating a maximum of twenty six and twenty amino acids in the CDR1 and CDR3, respectively (6), used to construct phage libraries. Screening of this library was performed to select SdAbs recognizing heme. SdAbs specificity was tested against heme *versus* other physiologic tetrapyrroles, revealing that the selected SdAbs recognize heme specifically. These SdAbs also did not recognize heme inserted into hemoproteins. This suggests that the selected SdAbs recognize specific heme epitopes not exposed by protein-bound heme. This notion is supported by the CDR1 sequences of these sdAbs, encoding putative heme binding motifs. We developed an enzyme linked immunoabsorbant assay (ELISA) using these SdAbs and allowing for the specific detection of "free heme" in biological samples. We are exploring whether these SdAbs can be used to modulate the biologic effects of free heme, such as to prevent the pathogenesis of immune mediated inflammatory diseases.

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OP23. Supercomplexes of Prokaryotic Aerobic Respiratory Chains

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Several evidences emerged suggesting that aerobic respiratory chains are organized in supercomplexes, which are considered advantageous to the efficiency of the oxidative phosphorylation process.

We have pursued a thorough characterization of *Escherichia coli* and *Bacillus subtilis* respiratory chains to assess the presence of such assemblies.

The *E. coli* aerobic respiratory chain is composed by at least six enzymes, type I and II NADH:quinone oxidoreductases, succinate:quinone oxidoreductase, and cytochromes bo_3, *bd*, and *bd*' oxygen oxidoreductases, that accomplish the transfer of electrons from the reducing substrates NADH and $FADH_2$ to oxygen, with energy conservation. In *B. subtilis*, type I NADH:quinone oxidoreductase is absent, and a different set of quinol: and cytochrome *c*:oxygen oxidoreductases, namely cytochromes b_6c_1, *bb*', *bd*, aa_3 and caa_3 are present.

BN-PAGE and *in-gel* activity detection, sucrose gradient analyses and mass spectrometry of digitonin solubilized membranes from wild type and respiratory chain mutant strains led to the identification of supercomplexes in the respiratory chain of these bacteria. The presence of such assemblies during bacterial growth, as well as the respiratory chain enzyme activities and gene transcriptional profile was globally analyzed, and correlation patterns were calculated.

Three supramolecular assemblies were identified and characterized in the respiratory chain of *E. coli*, a formate:oxygen oxidoreductase supercomplex containing the aerobic formate dehydrogenase (Fdo) and bo_3 and *bd*I oxygen oxidoreductases, a supercomplex composed by type I and II NADH:quinone oxidoreductases and a third supramolecular structure composed by succinate dehydrogenase and cytochrome *bd*' [1, 2]. Furthermore, an obtained integrative analysis suggested a strong positive correlation between the supercomplexes composition and the transcription profiles of *E. coli* respiratory chain genes. The investigation of *B. subtilis* supercomplexes is under progress.

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Keywords: Aerobic respiratory chain, *Escherichia coli*, *Bacillus subtilis,* Formate oxygen reductase supercomplex, Cytochrome b_6c_1:caa_3 oxygen reductase supercomplex, Succinate oxygen reductase supercomplex

Poster Presentation

PP1. Carriage of pneumococcal serotype 19A reduces *Staphylococcus aureus* carriage in Portuguese children in the era of PCV7

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Background and aims: An antagonism between colonization by pneumococcus and *Staphylococcus aureus* has been reported for both 7-valent pneumococcal conjugate vaccine (PCV7) serotypes (VT) and non-VT (NVT) [1-3]. We aimed to evaluate the contribution of individual serotypes to the antagonism.

Methods: Nasopharyngeal samples were collected from pre-school children before PCV7 was available (1996-1998, n=2,111) and when uptake of ≥1 PCV7 dose was around 70% (2006-2007, and 2009, n=2,100). Pneumococcal and *S. aureus* isolation and pneumococcal serotyping were done by standard procedures. A multiple logistic model was used to explore the association between pneumococcal and *S. aureus* carriage.

Results: Pneumococcal carriage was not affected by introduction of PCV7. NVT replaced VT, increasing from 53% in the pre-PCV7 period to 90% in the PCV7 period. Carriage of *S. aureus* increased from 14% to 17%. A negative association was observed between *S. aureus* and VT (OR=0.24, 95% CI 0.07-0.71) but not NVT (p=0.126) in the pre-PCV7 period and with both VT (OR=0.09, 95% CI 0.01-0.63) and NVT (OR=0.31, 95% CI 0.15-0.63) in the PCV7 period. Colonization by serotype 19A decreased colonization by *S. aureus* in the PCV7 period (OR=0.54, 95% CI 0.32-0.92). For other serotypes it was not possible to conclude on their impact on *S. aureus* carriage due to small sample size. To obtain statistical significance, serotypes were grouped based on the strength of the mean OR for both periods. A negative association was observed by grouping serotypes 5, 14, 19A, 19F, and 23F in the pre-PCV7 period (OR=0.16, 95% CI 0.04-0.567) and serotypes 6A, 6C, 14, 19A, 19F, 21, and 23F in the PCV7 period (OR=0.10, 95% CI 0.03-0.37). Addition of other serotypes did not change the results.

Conclusion: Carriage of serotype 19A decreased *S. aureus* colonization in the vaccinated population. Further studies are needed to confirm this ability for other serotypes.

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Keywords: carriage, co-colonization, pneumococcus, *S. aureus*, antagonisn, PCV7

PP2. Polymorphisms in Inc proteins and differential expression of *inc* genes among Chlamydia trachomatis strains correlate with invasiveness and tropism of lymphogranuloma venereum isolates

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Chlamydia trachomatis is a human bacterial pathogen that only multiplies within an intracellular membrane-bound vacuole, the inclusion. *C. trachomatis* ocular and genital infections are a significant clinical and public health problem worldwide. *C. trachomatis* includes ocular and urogenital strains usually causing infections restricted to epithelial cells of the conjunctiva and genital mucosa, respectively, and lymphogranuloma venereum (LGV) strains, which are sexually transmitted but can infect macrophages and spread into lymph nodes. In spite of these differences in tropism and invasiveness, the genomes of *C. trachomatis* strains exhibit >98% of identity at the DNA level. In this work, we studied whether *C. trachomatis* Inc proteins, showing a bilobal hydrophobic domain that may mediate their insertion in the inclusion membrane, could be a factor determining these different types of infection and tropisms. We performed phylogenetic and molecular evolution analyses, and used real time-PCR, to study if Inc proteins and the expression of *inc* genes varies among *C. trachomatis* strains. Analyses of polymorphisms of 48 Incs from 51 *C. trachomatis* strains representing the three disease groups revealed the presence of significant amino acid differences that were mainly due to variations between Incs from the LGV group and Incs from the ocular and urogenital group. Evolutionary dynamics analyses showed that 10 *incs* are likely under positive selection and indicated that most non-silent mutations are LGV-specific. Expression analyses in prototype and clinical strains representing each disease group identified 3 *inc* genes that showed LGV-specific expression. These results indicate that small variations in the amino acids of Inc proteins and in the expression of *inc* genes may contribute for the distinct tropism and invasiveness observed with *C. trachomatis* LGV strains.

Keywords: Host-Pathogen interactions, *Chlamydia trachomatis*, Type III Secretion, Inc proteins, Phylogenetic analyses, Gene expression

PP3. Screen for novel type III secretion effectors of *Chlamydia trachomatis*

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Chlamydia trachomatis are Gram-negative bacterial pathogens that cause ocular and genital infections in humans. These bacteria are genetically intractable and only multiply within an intracellular membrane-bound vacuole, the inclusion. *C. trachomatis* encode for a type III secretion (T3S) system, used by many bacterial pathogens to manipulate eukaryotic host cells by injecting effector proteins into their cytosol or membranes. We aim to screen for new effector proteins of *C. trachomatis* using *Yersinia enterocolitica* as surrogate host. We selected 22 genes that may encode uncharacterized effectors from the genome of *C. trachomatis* strain L2/434/Bu. We tested if these 22 *C. trachomatis* proteins were secreted as full-length proteins after expression in *Y. enterocolitica*. Of the 22 proteins analysed we observed that 11 proteins were secreted in a T3S-dependent manner. From these type III-secreted proteins, we focused our attention on CT142, CT143, and CT144, which are likely encoded within the same transcriptional unit, and analysed in more detail the presence of a T3S signal. Because T3S substrates normally possess a non-conserved and non-cleavable secretion signal within their first 15-30 amino acids, we constructed translational fusions comprising the first 20 amino acids of CT142, CT143, and CT144, and a reporter protein (the mature form of TEM-1 β-lactamase). We concluded that the first 20 amino acids of each protein tested were sufficient to drive the secretion of TEM1. However, the secretion of full length CT143 was more efficient than the respective fusion protein, suggesting that there should be an additional signal for secretion. We also raised antibodies against CT143 and analysed its kinetics of expression and localization in infected Hela cells. CT143 showed a peak of expression at around 30 hours post infection. By immunofluorescence, we observed that CT143 appears mostly as intra-inclusion dots that sometimes appear to overlap with the inclusion membrane.

Keywords: Chlamydia trachomatis, effector proteins, TypeIII secretion, host pathogen interactions

PP4. Nitrogen-fixing biofertilizers for gramineous crops

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The exploitation of biological nitrogen fixation is perceived as a sustainable way of improving crop yields, by supplying plant's needs of nitrogen while ameliorating environmental impacts and reducing the dependence on chemical fertilizers[1].

Large areas in southern Portugal are dedicated to "montado", an agro-forestry-pastoral ecosystem with important contribution to the Portuguese economy and ecology. The exploitation of cork is often associated to extensive cultivation of fodder crops, such as annual-ryegrass (*Lolium multiflorum*, Lam). The objective of this work is to search for native ryegrass-associated diazotrophs, aiming at the selection of efficient strains for subsequent use as biofertilizer. More than 200 diazotrophic bacterial isolates were recovered from the root external environment and annual-ryegrass tissues. Taxonomic identification was accomplished by restriction analysis of the 16S rRNA gene[3] and hierarchical clustering of the resulting profiles. Several genomic clusters were obtained which, by 16S rRNA gene sequencing of selected representatives, were found to be related with the genera *Pantoea*, *Serratia*, *Achromobacter*, *Paenibacillus*, *Microbacterium*, *Bosea*, *Sphingomonas*, *Rhizobium*, *Bacillus*, *Micrococcus*, and *Pseudomonas*. ERIC-PCR fingerprinting was used for identification of redundant isolates. The presence of the *nifH* gene, encoding the nifH subunit of nitrogenase, was screened[2] and the ability to grow in the absence of nitrogen was confirmed. Preliminary plant inoculation assays in nitrogen-free medium indicated some *Sphingomonas* isolates as able to supply the plants with fixed nitrogen. The library of diazotrophs was also screened for other plant-growth promoting activities, such as, *in vitro* production of phytohormones (auxins), solubilisation of mineral phosphate, hydrolysis of plant polymers (cellulose and pectin), production of siderophores and antifungal activity on the phytopathogenic *Phytophthora cinnamomi*. Many isolates (45%) were able to accomplish at least one of these activities. The most effective auxin producer was a *Pantoea* isolate; its effects on seed germination and early plant development are currently being evaluated.

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Keywords: biofertilizer, ryegrass, diazotrophic bacteria, non-symbiotic nitrogen fixation

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PP5. Zeb1 function in neural stem/progenitor cells – a genomic approach

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Current interest in neural stem cells derives from the prospect of using them in brain repair strategies, but also to understand neurodevelopmental pathologies. This will require, however, a significant improvement of our understanding of the gene expression programs associated with their maintenance and differentiation, and how these are regulated.

Several lines of evidence suggest a regulatory function for the zinc-finger transcription factor Zeb1 (also known as: Zfhx1a, TCf8 and Zfhep) in neural stem/progenitors during embryonic development. Zeb1 is highly expressed in proliferating neural progenitors located in various regions of the brain and spinal cord[1] and its down regulation occurs when cells migrate out of the germinal zone prior to differentiation, suggesting a functional role at the progenitor stage. Such a function is indeed supported by decreased proliferation in the germinal layers of the forebrain of Zeb1 null embryos, associated with ectopic expression of the cell cycle regulators p15Ink4b and p21Cdkn1a[2]. In spite of these observations, no serious attempts at understanding the function of Zeb1 in neural stem/progenitor cells have been made. Instead, most research has focused on its role in tumour development, where Zeb1 activation of an EMT program triggers cellular mobility and subsequent dissemination of cancer cells. Furthermore, it has been shown that by directly inhibiting members of the miRNA 200 family[3], Zeb1 is essential to overcome the repression of stem cell factors such as Sox2, Klf4 and Bmi1, inducing stem cell properties in invasive tumour cells that can initiate tumour growth at distant sites. Whether such "stemness" inducing mechanism is part of Zeb1 function in neural progenitors is currently not known.

We have recently started to address the function of Zeb1 in neural stem/progenitor cells by characterizing its transcriptional program genome-wide. We are using a combination of mouse genetics and genomics, whereby location analysis (by ChIP-seq) is associated with transcriptional profiling of both gain and loss-of-function models.

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Keywords: neural stem cells, Zeb1, transcriptional program

PP6. A reverse genetics approach to identify cardoon (*Cynara cardunculus*) genotypes with improved polyphenolic content

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Cynara cardunculus (*Cc*) is a Mediterranean species used as multipurpose crop. *Cc* is used as solid biofuel, green forage for ruminants, as a source of stalks for paper pulp, seeds for oil and flowers for cheese production. *Cc* has also a long traditional use in folk medicine. However, cardoon has other, still unexplored, potential for industrial use: the production of bioactive phenolic compounds. Plant polyphenols are compounds that play a positive role in well being, in improving health and controlling immune function. Polyphenols have low toxicity and antioxidant properties, and have been considered useful in the prevention and treatment of different human diseases, including cancer, cardiovascular and chronic disorders. Some polyphenolic compounds were already identified in cardoon and implicated in a wide range of biological processes with activity as antioxidant, antibacterial and antifungal. Preliminary results obtained at the host team (CEBAL) support a strong biological potential of cardoon phenolic extracts in human health. Results show a correlation between the phenolic content of *Cc* extracts and antioxidant activity, where leaves extracts exhibit the highest antioxidant and antitumural activity. This project will apply a reverse genetics approach to identify cardoon plants with allelic variations in genes (C3'H and HQT) involved in the phenylpropanoid pathway by High Resolution Melting (HRM) [1]. For this study, a representative collection of biological diversity of cardoon will be used. The cardoon plants identified with base mutation in the target genes will be evaluated for an improved biological activity. Total phenol content, antioxidant activity and antitumorigenic capacity will be determined in these plants. This project will contribute to elucidate about the molecular basis of the production of phenol bioactive compounds in *Cynara cardunculus*. Results of HRM will be presented.

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Keywords: Cardoon (*Cynara cardunculus*), antioxidant, antitumorigenic, phenylpropanoid pathway, C3'H, HQT, High Resolution Melting.

PP7. "Understanding salt tolerance in plants: Two different approaches"

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Around 20% of the worldwide arable land is affected by high salt concentrations [1]. This makes salt stress one of the major abiotic stresses affecting crop yield. Consequently, understanding the mechanisms underlying salt tolerance is very important. Here, we present two approaches to improve our knowledge on salt stress -an evolutionary study of two salt responsive gene families, and the identification of novel genes relevant for salt tolerance in rice (*Oryza sativa* L.).

Studying the evolution of individual gene families informs us how stress adaptation is achieved in plants. We focused on NHX and SOS1 transporter families due to their importance in salt tolerance. These proteins perform the compartmentalization [2] and the efflux [3] of Na_+, respectively. Our results [4] suggest that, while NHX proteins can be duplicated and subfunctionalized, they must retain their basic function and thus are under stronger purifying selection. In contrast, SOS1 proteins cannot be easily subfunctionalized and any duplicate copy seems to be preferentially lost, thus exhibiting more relaxed selective pressures. In conclusion, this shows that there are multiple mechanisms to achieve stress adaptation, in particular salt tolerance, through gene duplication events [4].

Additionally, rice is the staple food for more than half of the world's population and the most salt sensitive cereal [5]. For this reason, we selected this plant as a model to identify novel salt tolerance genes. Currently, we are performing a Genome Wide Association Study (GWAS) with 59 rice varieties, which were previously phenotyped under control (0dS/m) and salt (12dS/m) conditions [6]. Hereafter, we intend to follow-up studying the most interesting results.

In the future, by learning how stress adaptation occurs in nature and finding novel salt tolerance genes we expect to increase the basic knowledge on salt stress, and provide valuable information for crops marker assisted breeding.

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Keywords: stress adaptation, salt tolerance, gene duplication, gene neo/sub-functionalization, GWAS

PP8. Chickpea seed protein digestibility and antinutritional content

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In addition to their role in plant propagation, seeds are important sources of food, animal feed and industrial raw material. Grain legume seeds, also described as "poor man´s meat", are affordable staple foods with a balanced and rich nutritional composition (protein, minerals and vitamins [1]). Among the dry grain legumes, chickpea (*Cicer arietinum* L.) has one of the best nutritional compositions and ranks 3rd in world legume production [2]. Chickpea contains a number of bioactive substances with potential health-beneficial effects and for novel food formulations [3] still, reports on adverse reactions to its consumption are increasing. Although seed processing prior to consumption increases the seed nutritional value, it does not completely eliminate their antinutritional properties [3-5]. In addition, it was shown that seed nutritional value (and yield) can be negatively affected by growing conditions during seed development [6-8]. We aim to detect and characterize chickpea seed proteins with antinutritional potential from seeds produced in the field in two consecutive, but contrasting years. To fulfill this objective we will focus on proteins that are: i) resistant to boiling; ii) resistant to simulated digestion and iii) able to pass an *in vitro* intestinal absorption model [9]. Preliminary results show that, after cooking and simulated digestion, up to 36% of the initial material and 22% of the initial protein remain undigested. At the end of digestion a total of 14 resistant polypeptides were detected and 4 were identified and predicted as allergenic using the Algpred allergen prediction database (http://www.imtech.res.in/raghava/algpred). In this report progresses in seed processing, *in vitro* digestion, and in sample preparation for the proteomic studies are presented. The methodologies established will allow us to evaluate the effects of the growing conditions during seed development on the chickpea protein fraction. Our goal is to evaluate the protein profile in distinct genotypes.

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PP9. Differential expression of *Lathyrus cicera* and *Lathyrus sativus* transcriptomes in response to rust (*Uromyces pisi*) infection

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Chickling pea (*Lathyrus cicera* L.) and grass pea (*Lathyrus sativus* L.) have great potential among grain legumes due to their good adaptability to inauspicious environments, high protein content and resistance to serious diseases. Due to the need of increasing sustainability of agricultural production systems the interest in the conservation and utilization of *Lathyrus* genetic resources is rising. Nevertheless, due to its past underuse, just a few molecular breeding tools were developed specifically for *Lathyrus* species. Research on more important economically related legumes can to a certain extend contribute to surpass this limitation.
We have compared the leaf transcriptome profiles of two contrasting accessions each of *L. cicera* and *L. sativus* in rust (*Uromyces pisi*) infected and non infected conditions. For *L. cicera* a normalized cDNA library was created using Illumina sequencing. For *L. sativus* a semi-quantitative RNA-seq method was chosen in order to detect also variation in expression level.
These new data will allow bridging *Lathyrus* genomic information with model species and better studied related legume crops in order to develop molecular tools that will allow breeding these ancient crops interesting for modern farmers.
Results will be discussed.

Keywords: Lathyrus, Gene Expression Profiling, *Uromyces pisi*, Disease resistance

PP10. Using functional genomics for improved manufacture of virus-based biopharmaceuticals: Transcriptional profiling and metabolic engineering of retroviral vector producing cell lines

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Recombinant retroviruses comprise several valuable biopharmaceuticals such as gene therapy vectors, recombinant vaccines and replicative-competent particles for oncolytic therapy. However, their manufacture faces challenges in several fronts, particularly the low-yields, high non-functional particles content and the animal sera required in to sustain long-term productivity. Our limited knowledge on the biochemical pathways related to virus production has impeded cellular and process engineering efforts to enhance production performances. In this PhD project they were examined the transcriptome and metabolome profiles in two scenarios: a retrovirus producer cell establishment and virus production under serum deprivation. A systems approach was taken to integrate the data and gain new insights on the process, particularly, to find gene targets for metabolic engineering for improved cell productivities.
During a producer cell establishment 12 metabolic networks were found to be recruited and more than 30 genes identified as potential candidates for metabolic engineering. Media manipulation targeting these pathways resulted in productivity improvements from 1.3 to 2.1 fold when targeted individually and, when in combination, a synergistic effect resulting in a 6 fold increase. Two of these pathways were chosen for gene engineering, resulting in more than 20-fold improvement of vector productivity.
During a serum deprivation challenge, lipids, particularly cholesterol, were identified as the main component necessary for virus production. The characterization of cell lipid content using ^{13}C-NMR spectroscopy revealed differences in lipid biosynthetic capacity responsible for serum heterotrophy. These differences were scrutinized using transcriptional profiling and two key players were identified: AMPK and the down-regulation of lanosterol synthase, an enzyme of cholesterol biosynthesis machinery.
This project is currently proceeding through the development of a high-throughput screening method enabling a large-scale metabolic engineering study to test all the potential gene targets identified and their combination, in the pursuit of in-depth knowledge of the metabolic hotspots of viral replication and improved producing phenotypes.

Keywords: retroviral-based biopharmaceuticals; functional genomics; systems biology; metabolism; lipids

PP11. "Bioactivator" ionic liquids?

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Ionic liquids are a real alternative to the conventional solvents used in biocatalysis due to the possibility of designing them to be biocompatible and to be enhancers of biocatalytic processes [1]. The synergy between ionic liquids and enzymes offers the appropriate tools for the industrial transformation of natural or synthetic materials under mild reaction conditions.
The processes catalyzed by hydrolytic enzymes such as lipases and amylases are on the forefront of enzymatic research in sectors as varied as the petrochemical, pharmaceutical or food industries [2].
Initially, the work tackled the study of the enzymatic activity of different commercial lipases in the presence of ionic liquids. There are already several sets of data available in literature about the effect of ionic liquids in lipases [3]. Since most of the typical imidazolium-based ionic liquids entail deactivation of lipases, our efforts will be focused on the design of ionic liquids based on the cholinium cation and aminoacid-based anions. The choice of these ionic liquids is based on their biocompatibility, the possibility of stabilizing the enzyme or enhancing its activity through the interaction between aminoacids from the enzyme and those from the ionic liquids and the creation of buffer solutions with adequate pH avoiding the need of adding other compounds to the solution. The results show that the performance of enzymes in ionic liquid solutions depends on the match between the pH of the ionic liquid solution and the pH at which the enzyme is active.
Moreover, since the Information about the behaviour of □-amylases in the presence of ionic liquids is scarce [4], we intended to analyse the effect of ionic liquids on other kind of common commercial enzymes such as amylases. We plan to use a large set of distinct ionic liquids to interpret the effect of both the cation and the anion on the stability and activity of the enzyme. The results obtained for the activity of α-amylase in two different families of ionic liquids revealed that the size of the alkyl chains and the constituent ions of the ionic liquids decisively affect the biocatalytic potential of the enzymes. In general, cholinium-based ionic liquids allow higher levels of amilolytic activity than imidazolium-based ionic liquids.

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Keywords: enzyme, ionic liquids, activity, stability

PP12. DEVELOPING EVEN HIGHER IONICITY IONIC LIQUIDS (HIILS). SIMPLE: JUST ADD SALT

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Efforts to make liquid-liquid extraction processes more efficient and eco-friendly in the separation of azeotropic or close boiling point mixtures get a boost from the use of a relatively new class of compounds known as ionic liquids (ILs) [1]. These compounds are entirely composed by ions and are liquid due to the complex interplay between electrostatic and dispersive forces occurring between them [2,3].
This work exploits a new scientific branch of the ILs field, the increase of the Coulombic character of these compounds through the solubilisation of simple inorganic salts (ISs) in their milieu. This simple and inexpensive way to increase the ionicity of an IL can be achieved, at very low cost, while preserving the liquid nature of the new ionic media. The resulting purely ionic media —distinct ionic liquid plus inorganic salt mixtures— are liquid in an extensive concentration range and can be aptly denominated High Ionicity Ionic Liquids (HIILs). Thus, a new class of ionic liquids, HIILs, will emerge because the media is purely ionic and liquid.
The fundamental concept behind this work is to study the enhancement in the ionicity of an ionic liquid by the addition of an inorganic salt, and to test these mixtures as solvents in liquid-liquid extraction of azeotropic mixtures. To this end, the solubility of inorganic salts in ionic liquids will be studied, and the effect of the inorganic salt addition on the ionic liquids' most relevant thermophysical properties, such as density, viscosity, conductivity and diffusion coefficients. NMR studies were also used to study the occurrence of interactions and structural changes in the ionic liquids upon the addition of inorganic salt. Finally, the performance of the High Ionicity Ionic Liquid as extraction solvent was evaluated in the separation of heptane + ethanol azeotropic mixture and the results compared to those of the neat ionic liquid.

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Keywords: Ionic Liquids, Inorganic Salts, Ionicity, Separation, Azeotropic Mixtures

PP13. Systems-level analysis of baculovirus-host interactions: from genomic to metabolomic decomposition

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The Insect Cell-Baculovirus Expression Vector System (IC-BEVS) is a major biotechnological tool for the production of heterologous proteins, vaccines, virus like particles (VLPs) and, more recently, vectors for gene therapy. Nevertheless, the fundamental knowledge on this system is still underdeveloped. Viral infection induces profound alterations in the host cell, such changes being ultimately responsible for their performance in the bioprocess. Since cells are intricate networks of interactions, which are further complicated due to viral infection, only an integrated strategy will allow understanding cell physiology and profiting it to enhance the production potential of cells.

Two insect cell lines, *Sf*9 and *Hi*5, commonly used for the production of viral vectors and recombinant proteins, were used in this work. Sf9 cells show a higher capacity for virus replication, with titers 100 times higher than Hi5 cells. On the other hand, Hi5 cells show a better performance for the production of recombinant proteins, with productivities 3 to 4 times higher. Despite their common use in the biotechnological field, the differences in the complex nature of the cross talk between virus and host, which are behind such different productivities, have not been elucidated. Moreover, related cell lines show distinct phenotypes regarding protein processing pathways. Several analytic platforms (metabolomics, fluxomics and gene expression) were applied to shed light on the dynamics of baculovirus-insect cell interaction. The acquired data were integrated in a Systems Biology perspective for identifying system bottlenecks as metabolic engineering targets to optimize cell productivity and bioprocess development.

Keywords: Baculovirus; virus-host interactions; metabolomics

PP14. New CO_2 separation membranes based on pyrrolidinium ionic materials

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New strategies for the use of energy from renewable resources, non-fossil fuels and carbon capture and storage are being developed at a large scale. In particular, research targeted at developing new approaches for CO_2 separation has been undertaken by the scientific community. The use of ionic liquids (ILs) for CO_2 separation has received growing attention in recent years, not only due to their high levels of solubility and selectivity of CO_2 relative to other gases but also because of their unique intrinsic properties [1]. It has been shown that the best way to approach ILs for industrial gas separation is to use polymeric ionic liquids (PILs) to prepare membranes, because they present fundamental engineering and economic advantages [2].
In this work, CO_2 separation performances of a new series of polymeric ionic liquids composite membranes based on poly(diallyldimethylammonium) bis(trifluoromethylsulfonyl)imide, poly(pyr_{11}^+ NTf_2^-), by the addition of 0, 20, 40, 60, 80 and 100 wt.% of 1-Butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([pyr_{14}][NTf_2]), were measured in order to establish the feasibility of using these sustainable ionic materials as membranes for flue gas separation and natural gas purification. Additionally, in order to understand how the introduction of different anions can change the CO_2 separation performance of these materials, PILs with different anion mixtures were also synthesized and composite membranes with 20 wt.% of [pyr_{14}][NTf_2] were prepared and tested for CO_2 separation membranes.
Gas permeation experiments using CO_2, N_2 and CH_4 in all the prepared membranes were performed using a time-lag apparatus. The study of the whole material range allows for the selection of the appropriate amount of IL so that the gas separation membrane closest to the upper bound of the Robeson plot can be prepared. On top of that, the study of PIL membranes with different anion mixtures illustrates that is possible to tune their gas permeation properties by changing the PIL anion.

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Keywords: polymeric ionic liquid membranes, pyrrolidinium-based materials, time-lag, CO_2 separation

PP15. Awakening of silent secondary metabolites genes of two model Ascomycota by ionic liquids

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Fungal secondary metabolites (SMs) are structurally and chemically diverse low-molecular-weight molecules that can be considered either beneficial or prejudicial (*e.g.* penicillin and mycotoxins, respectively). The production of novel SMs under normal laboratory conditions presents great limitations, mainly because their biosynthetic gene clusters remain cryptic. One way to stimulate SMs production is to induce these metabolic pathways by supplementing the growth media with different compounds. Our team has recently observed that supplementation of the growth media with ionic liquids augments secondary metabolites production in several Ascomycota strains.[1,2]

The present study aims to better understand the effects of ionic liquids in fungal secondary metabolism by using whole genome transcriptome profiling. Bio-informatics analyses based on the highly conserved polyketide synthase and non-ribosomal peptide synthase domains have led to the identification of most SM biosynthetic gene clusters in fungal genomes.[3]

To achieve this goal we performed a comparison of the transcriptomic profiles (Affymetrix custom microarrays) of two model fungal species - *Aspergillus nidulans* (FGSC A4) and *Neurospora crassa* (FGSC 2489), grown either in minimal media or media supplemented with an ionic liquid. The selected ionic liquids were 1-ethyl-3-methylimidazolium chloride (toxic and recalcitrant to degradation) and cholinium chloride (benign and readily biodegradable).

The supplementation of the growth media with an ionic liquid increased the diversity of low-molecular-weight compounds in the media. This was correlated with higher expression of transcript levels of genes coding for the core biosynthetic enzymes of several polyketides and non-ribosomal peptides.

In *A. nidulans*, *laeA* codes for a histone-lysine methyltransferase which is considered important in the activation of some biosynthetic gene clusters. Despite *laeA* major down-regulation, a significant number of the backbone genes, putatively under LaeA regulation, were substantially up-regulated.

Advance studies will focus on the identification of novel SMs and the characterisation of the corresponding gene cluster regulation.

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Keywords: *Aspergillus nidulans*, *Neurospora crassa*, secondary metabolism, transcriptomics, gene clusters, ionic liquids.

PP16. Production of helper-dependent canine adenovirus type 2 (CAV-2) vectors for gene therapy: Impact of transcomplementing gene products on producer cell-line

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The manipulation of adenovirus vectors (AdV) has been extensively studied. However, little attention is being paid to their producer cell-lines; cells are selected according to virus yields, neglecting the expression profile of transcomplementing gene products underlying cell performance. All AdV require for their manufacturing and replication a cell-line that expresses the adenoviral E1 functions in trans. Furthermore, helper-dependent AdV also require a recombinase expression, such as Cre-recombinase, by the transcomplementing cell-line. However, Cre is reported to induce growth inhibition and apoptosis due to DNA damage.
Previously, we had show that expression of E1 had a crucial role on CAV-2 vectors production, namely on adenoviral replication and cell viability by the influence of E1A and E1B, respectively.
In this work, the impact of Cre on MDCK-E1-Cre cells and production of helper-dependent CAV-2 vectors was evaluated, by analysing several MDCK-E1-Cre cell-clones with different Cre levels. MDCK cells were selected since this is the only canine cell-line approved by regulatory agencies to manufacture biopharmaceuticals. Cell-culture (cell growth, metabolism and physiological state under oxidative stress conditions) and viral production (CAV-2 productivity and excision of helper vectors) features were evaluated in different MDCK-E1-Cre cell-clones.
Although Cre impaired cell growth, increasing susceptibility to oxidative stress injury, the typical productivity of CAV-2 vectors was maintained in MDCK-E1-Cre cells. Moreover, these cells efficiently prevented helper vector replication, with productivities of helper-dependent CAV-2 vectors similar to those obtained with human AdV.
These results demonstrate that MDCK-E1-Cre is a robust cell-line for the production of helper-dependent CAV-2. Future work aims at scaling-up the production process using stirred tank bioreactors to debottleneck HDV availability for preclinical and clinical assays.

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Keywords: Adenoviral vectors, gene therapy, producer cell-line, MDCK.

PP17. New ionic liquids from renewable resources

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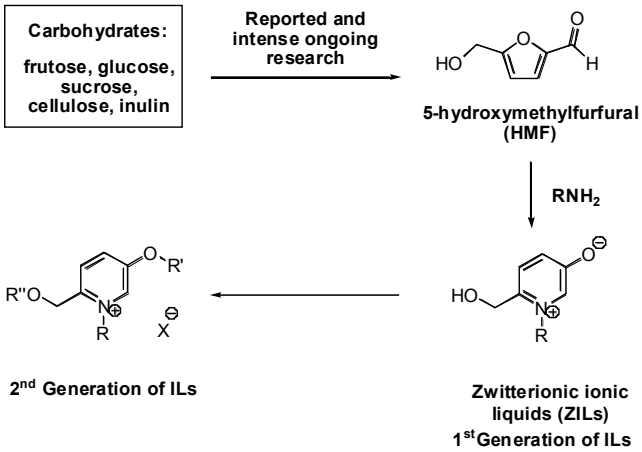
Fossil resources serve as feedstocks for the chemical and pharmaceutical industry producing a wide range of consumer goods ranging from cosmetics to plastics. With increasing consumption/dependence of fossil resources, biorenewable resources as an alternative are of considerable interest and great practical benefit. Biomass has attracted the attention of chemists as an important feedstock for the renewable production of fuels and bulk chemicals.[1] 5-Hydroxymethylfurfural (HMF) is an important bridge molecule linking biomass to fuels and chemicals, as it can be derived from renewable resources like fructose, glucose/cellulose and is very useful for the production of the biofuel dimethylfuran and other important commodities[2]. Recently, natural compounds have been used to prepare the cationic or anionic moiety of room-temperature ionic liquids[3]. Owing to their unique ability to dissolve most molecules including carbohydrates under relatively mild conditions, along with their existing advantages (e.g., as designable and recyclable solvents with low volatility and toxicity), ionic liquids (ILs), have attracted rapidly growing interest, particularly in the pursuit of renewable energy and sustainable chemicals. Here, we propose a new strategy to discover a new and competitive platform of ionic liquids containing the pyridinium cation[4] readily available from renewable resources HMF.



Using *n*-butylamine as the model substrate, the key transformation from HMF to the pyridinium cation was studied by screening different catalysts like bases, Lewis acids, inorganic salts and acids. From this optimization studies, we achieved efficient reaction conditions for this key transformation.

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Keywords: 5-hydroxymethylfurfural, biorenewable resources, ionic liquids, pyridinium, sustainability

PP18. Crz1 and Ca2+ - signaling in *Saccharomyces cerevisiae* exposed to arsenic Stress

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Arsenic is a highly toxic metalloid widespread in the environment. To cope with arsenic stress, organisms have developed a myriad of protection mechanisms including arsenic detoxification and the maintenance of redox homeostasis disturbed by arsenic compounds. In this work we show that supplementation of media with Ca2+ enhances tolerance of wild type and arsenic-sensitive *yap1*mutant strains exposed to arsenic. Crz1 is essential in this process since its disruption impairs the acquisition of tolerance mediated by Ca2+ as well as confers a moderate degree of sensitivity to the cells subjected to the metalloid. In addition, our work provides evidences that arsenic elicits a transient increase of free cytosolic Ca2+, which in turn leads to Crz1 nuclear compartmentalization, and culminates with the transcriptional activation of the cell wall biosynthetic gene *GSC2* and genes encoding Ca2+ transporters, such as *PMR1* and *PMC1*. Besides, the disruption of the Ca2+ import system involving Mid1/Cch1 compromises the acquisition of cellular tolerance to arsenic. Taken together, these data establish, for the first time, that activation of Crz1 and Ca2+-signalling pathways contributes to yeast adaptation under arsenic stress conditions.

PP19. Structural Virology Of The Murid Gamma-Herpesvirus 4

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Capable of establishing lifelong latency in the host, Herpes viruses are a striking cause of human viral disease. The murid herpesvirus 4 has been widely explored as a model for research on the human gamma-herpesvirus pathogenesis due to its ability to infect mice via the nasal passages and also because it is genetically related to other malignancy-associated pathogens such as the human Epstain-Barr virus (EBV) and Kaposi's Sarcoma herpesvirus (KSHV) [1, 2]. KSHV has an etiologic role in Kaposi´s sarcoma, the leading AIDS malignancy disorder, and in certain lymphomas whilst EBV is also associated with lymphomas and nasopharyngeal carcinoma. As with EBV, MuHV-4 establishes a lifelong latency stage as a multicopy episome in the nucleus of host B-cells and only a small fraction of viral genes are expressed. Amongst this fraction of genes, an ORF protein, ORF73, was demonstrated to promote a deficit on the establishment of latency in vivo when mutant viruses failed to express them [3]. ORF73 is a nuclear protein with remarkable sequence homology with KSHV latency-associated nuclear antigen (LANA) and striking predicted structural homology with the C-terminal domain of the EBV nuclear antigen 1 (EBNA1), mutually crucial for viral latency by tethering the viral episome to host chromosomes, therefore ensuring replication and segregation of the viral genome to daughter cells [4, 5]. C-terminal LANA and EBNA1 were described to bind terminal repeat (TR) DNA of the viral genome upon dimerization, suggesting that C-terminal of ORF73 may also be involved in episome maintenance functions [6, 7]. Additionally, an unconventional SOCS-box motif in the C-terminal of ORF73 was also shown to be involved in the binding of several cellular and viral proteins to modulate their transcription and affect cellular growth through E3-ubiquitin ligase activity [8]. Successful cloning, expression, purification and crystallization allowed us to determine the first X-ray crystallographic structure of the DNA-binding domain of the murid ORF73 protein.

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Keywords: herpesvirus, latency, ORF73, crystallography.

PP20. Unveiling specificities of the substrate pathways in a high activity H_2 producing enzyme

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Hydrogenases are metalloenzymes able to catalyze the reversible reaction $H_2 \leftrightarrows 2H^+ + 2e^-$ without the use of overpotentials or noble metals, an ability that as intrigued scientists for many years. In the enzymes of the [NiFe] class the active site is constituted by one nickel and one iron ions [1]. Usually, the nickel is coordinated by four cysteines and the iron by two of the previous cysteines, two CO molecules and one CN^- ion. Since the active site is deeply buried these enzymes need to have channels for H_2 diffusion and as well as proton pathways connecting the active site and the exterior of the protein. These pathways have been shown to influence the catalytic properties of these enzymes.
The [NiFeSe] hydrogenases are a subgroup of the [NiFe] family especially interesting due to their higher activities [2]. In these hydrogenases one of the cysteines coordinating the nickel is replaced by a selenocysteine. The connection between the different activity of these hydrogenase and the presence of the selenocysteine is not clear, and there may be other factors that can contribute to this property.
In order to unveil these other factors, we studied H_2 diffusion and proton pathways in the [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* Hildenborough, using computational methodologies, and compared them to the ones previously characterized in the standard [NiFe] hase from *Desulfovibrio gigas* [3, 4].
In the [NiFeSe] hydrogenase, we found an alternative channel that allows for the direct access of H_2 to the active site and is absent in standard [NiFe] hydrogenases, which could be related to the higher activities observed for [NiFeSe] hydrogenases [5]. We also concluded that the proton transfer pathways of [NiFeSe] hases and [NiFe] hases are located in the same region of the protein, but the residues composing them are quite different [5].

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Keywords: hydrogenase, selenocysteine, H_2 diffusion, proton pathways

PP21. Structural investigation of the NO sensing domain of the human soluble guanylate cyclase

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Soluble guanylate cyclase (sGC) is a heterodimeric, nitric oxide (NO)-sensing hemoprotein [1]. A heme cofactor is localized in the H-NOX domain [2]. sGC is responsible for the catalytic conversion of GTP to cGMP [2,3, 4]. NO-sGC-cGMP-dependet pathway is completely inactivated in the case of heme oxidation and the subsequent heme loss. In those cases reactivation of the enzyme can be achieved by Cinaciguat or BAY 58-2667 which is one such molecules that is in clinical trials for treatment of Acute Decompensated Heart Failure [5]. Despite the interest in H-NOX domain-BAY bound complex, the structure of the human sGC complex has not yet been elucidated. Our goal is to pursue such studies by NMR or crystallography. Heterologous expression (BL21-DE3) efficiency was tested as a function of various conditions, temperature (17, 20 and 37 ˚C) and medium culture type (LB, 2xYT and TB modified). Higher yields of apo-protein were obtained in TB medium (17 and 20 ˚C). The samples from 2xYT and TB were then purified with a combination of His-tag affinity and anionic chromatographies. sGC expressed in TB yielded approximately 20 mg/L of apo protein while in 2xYT the yield was around 10.6 mg/L. Based on the elution time from the size exclusion (SE) column, we concluded that the resulting apo-protein sample was in the form of a soluble high order-polymer. Incubation of the apo protein in its polymeric form with BAY 58-2667 results in the formation of the monomeric form. In addition, 1D NMR analysis showed a well folded H-NOX domain-Bay monomeric form. These preliminary results contribute significantly for the establishment of the biophysics properties of this construct. In the near future, we wish to further optimize protein production in minimal medium to label the protein with ^{15}N and $^{13}C/^{15}N$ for NMR spectra analysis.

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Keywords: Hemoprotein, Heart Failure, NMR, Protein Structure.

PP22. Study of energy transduction in bacteriorhodopsin using continuum electrostatics methods

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Bacteriorhodopsin (bR) is a light-driven proton pump, and a well characterized model system for energy transduction. In response to luminous stimuli, the retinal chromophore of bR isomerizes and triggers a series of protonation/deprotonation events and conformational changes that lead to the transfer of protons from the cytoplasmic to the extracellular side of the membrane.[1] This pumping of protons generates an electrochemical proton gradient across the membrane, which is known to affect the pumping rate of the protein. bR has been extensively studied both experimentally and computationally, although, among the different theoretical studies performed, only a few have considered the effect of a pH gradient.[2,3]
We have modified our continuum electrostatics method in order to include a pH gradient, and observed the effect of this gradient on the titration behavior of bR, particularly regarding the residues which are known to participate in the proton transfer process.
bR was simulated as a trimer inserted in a 341-DMPC lipid bilayer using molecular mechanics/dynamics (MM/MD)[4] methods. All titratable sites of bR were considered to be connected to either the cytoplasmic or the extracellular side of the membrane, which were exposed to different pH values. We then analyzed the effect of the pH gradient on important residues by calculating their two-dimensional titration curves as function of the pH on each side of the membrane.

References:
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