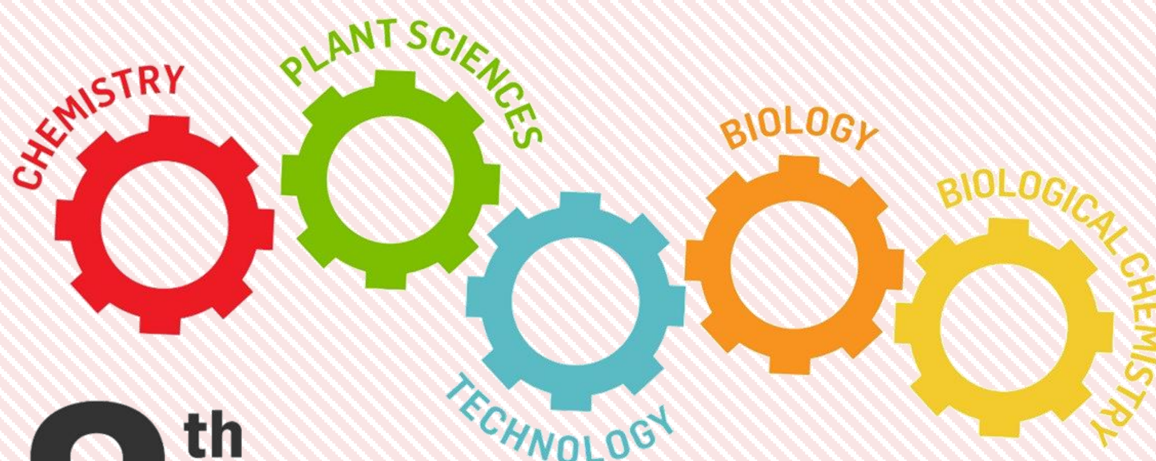
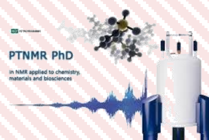


9th ITQB NOVA PhD Students' Meeting

7th - 9th Nov 2018

BOOK OF ABSTRACTS



Welcome to the 9th ITQB NOVA PhD Students´ Meeting

Dear Colleagues,

The Organizing Committee would like to welcome you to the 9th ITQB NOVA PhD Students´ Meeting.

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

This year, more than 60 students will be presenting their work in form of oral and poster presentations. Those presentations will cover a broad spectrum of scientific areas – ranging from Technology, Chemistry, Biological Chemistry and Biology to Plant Sciences.

In addition, we welcome you to the talks of our invited speakers. They are experts in various fields of science, differing from ITQB´s research lines to bring you in contact with different, exciting research topics. This year we have three invited speakers, João Correia (Marine Resources), João Lousada (Space Feasibility Concepts) and Joana Sá (Decision-making Process). All of them will surely inspire and motivate us to move forward with our research and careers.

The Organizing Committee wishes you a great 9th ITQB NOVA PhD Students´ Meeting.

The Organizing Committee of the 9th ITQB NOVA PhD Students´ Meeting:

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Ana Quendera

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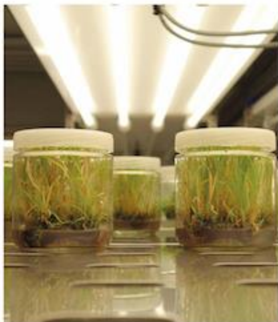

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Programme

PhD MEETING ITQB NOVA	WEDNESDAY 7TH NOV	THURSDAY 8TH NOV	FRIDAY 9TH NOV
9:00 – 9:20	REGISTRATION		
9:20 – 9:40			
9:40 – 10:00	OPENING SESSION	MARIA ASSUNÇÃO	NICOLE VIEIRA
10:00 – 10:20		JOANA RODRIGUES	ANA FERNANDES
10:20 – 10:40	ELEONORA MARINI	TIAGO JORGE	AGOSTINHO ALEXANDRE
10:40 – 11:00	PEDRO FERNANDES	MARIA GONZÁLEZ	MARA PINTO
11:00 – 11:20	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK
11:20 – 11:40	ARISTIDES MENDES		RAQUEL SANTOS
11:40 – 12:00	INÊS TORCATO	HÉLIA SALES	MARCOS SOUSA
12:00 – 12:20	AMAURY FASQUELLE	ANA LEAL	ANA PADUA
12:20 – 12:40	LUNCH	SARA COLANERO	LUNCH
12:40 – 13:00		LUNCH	
13:00 – 14:00			
14:00 – 14:20	CÁTIA BÁRRIA	JOÃO LOUSADA *Journey to Mars*	JOANA SÁ *Data Mining for Decision-Making*
14:20 – 14:40	JOSÉ RICARDO		
14:40 – 15:00	RICARDO SANTOS		
15:00 – 15:20	RITA BORBA	FRANCISCO LEISICO	DIEGO BORGES
15:20 – 15:40	COFFEE BREAK	BÁRBARA ABREU	PEDRO PEDROSA
15:40 – 16:00	CELSO MARTINS	COFFEE BREAK	POSTER SESSION BEER & SNACKS aralab
16:00 – 16:20	CATARINA FLORINDO	FILIPA CALISTO	
16:20 – 16:40	JOÃO CORREIA *Sharks, Mosquitos and Rock & Roll!*	VIVIANA CORREIA	
16:40 – 17:00		DAVIDE CRUZ	
17:00 – 17:20		SÓNIA ZACARIAS	
17:20 – 19:00	POSTER SESSION WINE & CHEESE		DINNER 20H CARAVELA D'OURO

SESSIONS

- PROBING BACTERIAL STRATEGIES
- THE LIFE'S CODE
- RESCUING THE PLANET
- PLANT THE FUTURE aralab
- MOLECULAR MACHINES IN ACTION
- GREEN CHEMISTRY
- ADVANCES IN BIOTECHNOLOGY
- MOLECULAR MEDICINE

The Venue

The 9th ITQB NOVA PhD Students' Meeting will be held at ITQB NOVA's ground floor. The registration desk will open on Tuesday 6th November at 15:00pm in the main entrance hall. Students' and invited speakers' presentations will be held in the auditorium. Poster sessions will take place in room 2.13. Coffee breaks will be held in room 2.13 as well as in the main entrance hall.

The Dinner

The Meeting´s Dinner will be on Friday, 9th November at Caravela D´Ouro in Algés, starting at 8:00 pm. The dinner is sponsored by Câmara Municipal de Oeiras. During dinner, best oral and poster presentations will be awarded and the money raised during registration will be donated to SEMEAR, a sustainable program of social inclusion for children, young people and adults with intellectual and developmental difficulties.

INVITED SPEAKERS

JOÃO CORREIA

Founder & Manager of Flying Sharks

Professor at Escola Superior de Turismo e Tecnologia do Mar

Education officer at Neptuno

info@flyingsharks.eu | joao.correia@ipleiria.pt

joao.correia@apece.pt | joao.correia@neptuno.pt

Talk Title: Sharks, Mosquitos and Rock & Roll!

What to expect:

Prepare for a presentation like you have never seen before… It is about an unusual life story… Adventures with sharks in the Bahamas, nocturnal injections in sharks at the Lisbon Zoo, cargo planes "filled" with animals for Oceanário de Lisboa, marine biology research and classes, enterprise management, a strong Star Wars influence and other unexpected surprises…

Short Biography:

João Correia has a Bachelor's degree in Marine Biology (University of Algarve, 1994), a Master's degree in Modulation of Marine Resources (Instituto Superior Técnico, 1997) and a PhD in Commercial Fishing of Sharks and Rays in Portugal (University of Aveiro, 2009). He received the award "Prémio do Mar Rei D. Carlos" for his PhD thesis.

Since a very young age, João has developed a special fondness for the sea, in particular for sharks. His professional career began in the Lisbon Zoo, where he was responsible for taking care of two sharks. Afterwards, he investigated sharks from the depths of the Portuguese coast at Instituto Português de Investigação Marítima. During the pre-opening of Oceanário de Lisboa and for the following eight years, João was responsible for the acquisition of all animals, including their capture.

He is the founder and general manager of Flying Sharks since 2006, an enterprise responsible for the transportation of live marine animals to the whole world and that provides consulting services. João also teaches at Escola Superior de Turismo e Tecnologia do Mar.

João has already published dozens of articles, book chapters and more than fifty scientific communications in international congresses, including two Best Presentation awards, one in 2012 in Chicago and another in 2013 in Bristol. He has already presented two TEDx talks and more than a hundred of presentations about sharks' biology and their conservation. His life story is portrayed in a book trilogy entitled "Sex, Sharks and Rock & Roll", written by him.

João Lousada

Systems Flight Control Engineer at International Space Station

Member of Space Generation Advisory Council (SGAC)

Co-Lead of the Space Safety and Sustainability project group

jmclousada@gmail.com

Talk Title: Journey to Mars

What to expect:

Mars is one of the most interesting places in our Solar System when it comes to looking for life outside of our planet. In the past, Mars had very similar conditions to those of Earth, with a moderate climate and liquid water, that could have sustained life as we know it. Whether life really developed on Mars and whether it might still exist, is one of the main reasons to explore the red planet. But Mars comes with many challenges that humankind has not faced before and in order to overcome them we need to study and learn as much as we can about them, here on Earth. The OeWF does exactly that: looking for places on Earth that are similar to Mars and testing different technologies and experiments that we might use on Mars.

Short Biography:

João graduated from Instituto Superior Técnico, in Portugal, with a Masters in Aerospace Engineering that included studies at Universitat Politecnica de Catalunya, in Spain, and University of Victoria, in Canada. He has worked in space feasibility concepts at the German Aerospace Agency (DLR) and in satellite assembly, integration and testing at OHB System, for European Space Agency (ESA) projects. Today he is a Systems Flight Control Engineer (STRATOS) for the Columbus Module of the International Space Station. He is also an active member of multiple volunteer organizations related to the space sector, such as the Space Generation Advisory Council (SGAC), where he has been participating and leading several technical projects as well as acting as co-lead of the Space Safety and Sustainability project group and as National Point of Contact for Portugal. João has also been a part of the core organizing teams of several SGAC events, including national, regional and global events; and recently he was elected SGAC regional coordinator for the European region. He is also an analog astronaut and field commander at the Austrian Space Forum, having taken in multiple analog missions with different space suit simulators.

JOANA SÁ

Associate Professor at Nova School of Business and Economics

Leader of Science and Policy (S&P) research group at Instituto Gulbenkian Ciência (IGC)

mjsa@igc.gulbenkian.pt

Talk Title: Data Mining for Decision-Making – From disease forecasting to political arguments

What to expect:

Every day we generate large amounts of data. Just by going online or using our cell phones, we leave informative traces, the so called "digital breadcrumbs", which can give away a lot of information about our individual actions. And, when this individual data is gathered and analyzed, it can be very revealing of both individual and global behavior patterns. At the S&P group we ask whether we can use some of these aggregated, anonymized data not only to know more, but also to make better decisions, both at the governmental and citizen levels.

During the presentation I'll focus on how we are analyzing some of these "breadcrumbs" to detect the onset of epidemic diseases, such as the flu, to help predict how many people will show up at the hospital on a given day. I will briefly discuss how we can use similar tools to understand sexual behavior, track non-infectious diseases, and even help keep our politicians in check.

Short Biography:

Joana Gonçalves-Sá is an Associate Professor at Nova School of Business and Economics. There, she also leads the Science and Policy (S&P) research group, that uses a computational and complex systems approach to improve decision-making, particularly related to health policy. She is also the director of the Graduate Program Science for Development, a PhD program in the life sciences, dedicated to scholars from the Portuguese-speaking African countries and East-Timor, at the Instituto Gulbenkian de Ciência. JGS was trained in Physics Engineering at Instituto Superior Técnico, Portugal, having worked on projects in the interface between mathematics, physics and biology. In 2003, she enrolled in the Gulbenkian PhD Programme in Biomedicine and did her PhD thesis work, at Harvard University, USA, on Systems Biology.

ORAL PRESENTATIONS

O1. A sporulation signature protease is required for spore assembly, germination and host colonization in *Clostridium difficile*

[1]*Marini, E., [3]Aguirre, A.M., [2]Ingle, P., [1]Melo M.N., [2]Minton, N.P., [3]Sorg, J.A., [1]Serrano M. & [1]Henriques, A.O.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República EAN, 2780-157 Oeiras, Portugal; aoh@itqb.unl.pt*
[2]*Clostridia Research Group, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), School of Life Sciences, University of Nottingham, UK;*
[3]*Texas A&M University, Department of Biology, College Station, TX, USA.*
**lead presenter: eleonora@itqb.unl.pt*

A genomic signature for endosporulation contains a gene coding for a protease, YabG, which in the model organism *Bacillus subtilis* is required for proper assembly of the spore coat. In both *B. subtilis* and in *Clostridium difficile*, a strict anaerobe, YabG is produced late during spore development under the control of the mother cell-specific regulator σ^K and associates with the spore surface layers. We show that YabG has a central domain that resembles the receiver domain of response regulators. This domain is atypical in that a glycine replaces the canonical phospho-acceptor aspartate, suggesting a phosphorylation-independent function. Atypical response regulators (ARD) function in directing protein sub-cellular localization and aid in bacterial organelle assembly. We present evidence that YabG is a cysteine (thiol) protease. We show that the substitution of Cys207, conserved among YabG orthologues and located within the ARD, by an Ala, eliminates an auto-proteolytic activity that self-limits the assembly of YabG, as well as processing of two spore germinant receptors. Moreover, residues His161 and Asp162, that together with Cys207 are superimposable on the catalytic triad of papain, are also required for YabG auto-proteolysis. An in-frame deletion of *yabG* or the C207A substitution both lead to the production of spores with misassembled surface layers that are more permeable to lysozyme and impaired in germination and host colonization. The activity of YabG is thus required for proper spore assembly and function. YabG is a novel type of protease whose function in the assembly of the spore surface layers is conserved across evolutionary distance.

Keywords: *sporulation; endosporulation signature; spore coat assembly; cysteine protease; YabG*.

***Acknowledgements**: This work was financially funded by the European Union Marie Sklodowska Curie Innovative Training Networks, CLOSPORE [642068].*

O2. Analysis of the cell cycle in *Staphylococcus aureus*

[1*]Fernandes, P.B., [1]Monteiro, J.M., [1]De Bakker, V. & [1]Pinho, M.G.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, Estação Agronómica Nacional, Oeiras*
*<u>*lead presenter</u>: pedro.fernandes@itqb.unl.pt*

Staphylococcus aureus is a pathogen that divides in three orthogonal planes over three consecutive division cycles [1]. Morphological changes during the cell cycle had remained elusive until the recent development of super-resolution microscopy techniques. We have characterized the different phases of the cell cycle of *S. aureus,* and found that cells elongate, in a process that requires the activity of peptidoglycan synthesis machinery and hydrolases. At the end of cell division, the mother cell is split in two daughter cells, concomitantly with the reshaping of the flat septal surface into a curved structure, which becomes approximately one hemisphere of each daughter cell. This process requires both the activity of peptidoglycan hydrolases and turgor pressure.
The biochemical and morphological changes that occur during the cell cycle, imply that cells are not identical in all cell cycle phases. Therefore, the cell cycle can be considered a source of variability in isogenic populations [2]. We therefore tested if expression of the Cell Wall Stress Stimulon (CWSS), a set of genes that mediate a coordinated response triggered upon cell wall damage [3], was homogeneous in an isogenic population of *S. aureus*. We concluded that CWSS activation was heterogeneous, but there was no apparent correlation between activation levels and antibiotic tolerance.

Keywords: Cell cycle; Heterogeneity; Antibiotic tolerance.

References:

[1] Pinho M. G., Kjos M. & Veening J.-W. How to get (a)round: mechanisms controlling growth and division of coccoid bacteria. Nat. Rev. Microbiol. 11: p. 601–614 (2013)
[2] Soltani M., Singh A. Effects of cell-cycle-dependent expression on random fluctuations in protein levels. Royal Society Open Science, 3 (12), art. no. 160578 (2016)
[3] Utaida, S., Genome-wide transcriptional profiling of the response of Staphylococcus aureus to cell-wall-active antibiotics reveals a cell-wall-stress stimulon. Microbiology, 149(10): p. 2719-2732 (2003)

***Acknowledgements**: This work was financially funded by Fundação para a Ciência e a Tecnologia (BD/119996/2016).*

O3. *Clostridium difficile* and Carbapenem Resistance

[1]*Mendes, A.L., [1]Covas, G., [2]Isidro, J., [1]Ventura, R., [2]Oleastro, M., [1,3]Filipe, S.R., [1]Serrano, M. & [1]Henriques, A.O.

[1]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal*
[2]*Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal*
[3]*Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal*
**lead presenter: al.mendes@itqb.unl.pt*

Clostridium difficile has an unusual peptidoglycan (PG), in that over 70% of the cross-links occur between the residues present in the third position of the stem peptides (3'-3'-type). These cross-links are formed through the action of L,D-transpeptidases (L,D-TPases). In contrast, the canonical D,D-transpeptidases (D,D-TPases) catalyse 3'-4'-type cross-links found in most bacteria. L,D-TPases are inhibited by carbapenems, a class of β-lactams, while D,D-TPases (penicillin-binding proteins) are inhibited by all classes of β-lactams. Carbapenems such as imipenem (IMP), are currently widely used in hospital settings to fight infections caused by Gram-negative bacteria. The emergence of carbapenem-resistant *C. difficile* strains thus poses a threat.

We characterized two strains of *C. difficile* from ribotype R017 that show high level resistance to IMP. We show that in these strains: i) the D,D-TPases PBP1 and PBP3 show amino acid substitutions thought to confer resistance to the antibiotic; ii) β-lactams of different classes induce synthesis of a class D β-lactamase (present in strains of other ribotypes) and an extra PBP (exclusively found in R017 strains), termed PBP5, which does not bind a fluorescent carbapenem. We also show that fluorescent vancomycin (Van-FL) which stains D-Ala-D-Ala motifs in PG, labels mainly the division septum, suggesting that the D,D-TPases are essential for the synthesis of PG during cell division and that the L,D-TPases synthesise the PG along most of the cell. In the presence of IMP, a susceptible strain forms shorter and curved cells consistent with defective cell elongation. Furthermore, in resistant strains growing in the presence of IMP, Van-FL now decorates the entire cell contour. This suggests that the mutant D,D-TPases now ensure PG synthesis along the entire cell. Carbapenem resistance in *C. difficile* thus shows three components: mutations in the D,D-TPases, and the antibiotic-induced production of an extra PBP and a β-lactamase.

Keywords: *Clostridium difficile*; antibiotic resistance; imipenem; peptidoglycan; microscopy.

***Acknowledgements**: This work was financially funded by Fundação para a Ciência e a Tecnologia (BD/105738/2014).*

O4. The discovery of novel autoinducer-2 receptors

[1,2*]Torcato, I.M., [3]Kasal, M.R., [1]Miranda, V., [1]Ventura, M.R., [3]Miller, S.T. & [2]Xavier, K.B.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
[2]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 2780-156 Oeiras, Portugal
[3]Department of Chemistry and Biochemistry, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081, USA
**lead presenter: inestorcato@itqb.unl.pt*

Quorum sensing is a cell-to-cell communication mechanism that allows bacteria to regulate group behaviors in function of their population density. More specifically, autoinducer-2 (AI-2) mediated quorum sensing is involved in inter-species communication and has been shown to regulate behaviors like biofilm formation and virulence factors expression according to the species composition of the community. Thus, to understand the molecular mechanisms underlying the inter-species communication in complex, multi-species environments it is essential to identify AI-2 receptors. Although there are several reports of different bacterial species regulating group behaviors in response to AI-2, until now only two type of AI-2 receptors are known: LuxP and LsrB. Here, we demonstrate that *Clostridium saccharobutylicum* possess a novel, non-canonical LsrB-like receptor. The crystal structure of this receptor showed that although it has two previously unobserved variations in the AI-2 binding residues it is still able to bind the same form of AI-2 as the canonical LsrB receptors. Through isothermal titration calorimetry we determined that the affinity of this receptor to AI-2 has a constant of dissociation in the submicromolar range. Mutagenesis studies allowed us to conclude that other compositions of the binding site are able to accommodate AI-2. These findings were fundamental for the identification of novel non-canonical LsrB receptors in bacterial strains where no AI-2 mediated quorum sensing was known. In parallel, a new chemically-based strategy to pull-down unknown AI-2 receptors from bacterial cell extracts was optimized. Altogether, this work provides relevant developments in the discovery of AI-2 receptors and is a key step towards the unraveling of the mechanisms through which AI-2 mediates bacterial behaviors.

Keywords: Quorum sensing; autoinducer-2; LsrB receptors; *Clostridium saccharobutylicum.*

***Acknowledgements**: This work was financially funded by Fundação para a Ciência e a Tecnologia (BD/105736/2014).*

O5. Functional Characterization of Two Forespore-Specific Paralogous Proteases of *Clostridium difficile*

[1*]Fasquelle, A., [1]Serrano, M. & [1]Henriques, A.O.

[1]*Instituto de Tecnologia Química e Biológica – António Xavier, Universidade Nova de Lisboa, Av. da República EAN, 2780-157 Oeiras, Portugal; aoh@itqb.unl.pt*
**lead presenter: amaury@itqb.unl.pt*

Clostridium difficile is an obligate anaerobe intestinal pathogen causing diarrhea and potentially lethal colitis. Sporulation is a cell differentiation program in which two cells, the forespore and the mother cell, cooperate to form a spore. In *Bacillus subtilis*, a signalling protease produced in the forespore, SpoIVB, is essential for the activation of a late mother cell-specific sigma factor, produced as a pro-protein, pro-σ^K[1]. However, this protease has a second, uncharacterized function in sporulation unrelated to the activation of pro-σ^K [2]. Remarkably, in *C. difficile*, in which σ^K is produced without a pro-sequence, the genome codes for two SpoIVB paralogues: SpoIVB1 and SpoIVB2.
We show that a *spoIVB1* in-frame deletion mutant produces 10 times less spores than the wild type strain, while a *spoIVB2* in-frame deletion mutant does not produces heat resistant spores. Using the $SNAP^{Cd}$ reporter we show that the expression of both genes is confined to the forespore and detected mainly after engulfment completion. Transcription of *spoIVB1* requires σ^F, while transcription of *spoIVB2* requires σ^G. Using phase contrast and fluorescence microscopy we show that the two mutants enter the sporulation pathway, form an asymmetric septum, but are blocked at the end or just after engulfment completion, consistent with the expression data. Importantly, thin sectioning transmission electron microscopy suggests that SpoIVB2 has a role in the formation of the spore cortex peptidoglycan.
Because of the central role of the spore in pathogenesis [3], and given their essentiality for sporulation, the two *C. difficile SpoIVB* paralogues may turn out to be good targets for the development of new strategies aiming at stopping infection, transmission and recurrence of the disease.

Keywords: *Clostridium difficile*; sporulation; cell-cell signaling.

References:

[1] Dong TC, Cutting SM. SpoIVB-mediated cleavage of SpoIVFA could provide the intercellular signal to activate processing of Pro-sigmaK in Bacillus subtilis. Mol Microbiol. 2003 Sep;49(5):1425-34.
[2] Oke V, Shchepetov M, Cutting S. SpoIVB has two distinct functions during spore formation in Bacillus subtilis. Mol Microbiol. 1997 Jan;23(2):223-30.
[3] Rineh A, Kelso MJ, Vatansever F, Tegos GP, Hamblin MR. Clostridium difficile infection: molecular pathogenesis and novel therapeutics. Expert Rev Anti Infect Ther. 2014 Jan;12(1):131-50.

***Acknowledgements**: This work was financially funded by the European Union Marie Sklodowska Curie Innovative Training Networks, CLOSPORE [642068].*

O6. Can you translate? *Streptococcus pneumoniae* RNase R effect in ribosome dissociation compromises protein synthesis

[1*]Bárria C., [1]Domingues S., [1]Arraiano CM.

[1] *ITQB NOVA, Oeiras, Portugal*
lead presenter: catiabarria@itqb.unl.pt

Ribosomes are molecular machines that carry out protein synthesis through a process termed translation. They are present in both prokaryotic and eukaryotic cells and their function is crucial for cell survival. In bacteria, the 70S ribosome is composed of two subunits, a small 30S subunit and a large 50S subunit. Each subunit is composed of ribosomal RNA and proteins and a proper assembly of these players is critical to form the active 70S ribosomal particle. After each round of translation, ribosome recycling is essential for reinitiating protein synthesis. Several translation factors are involved in ribosome recycling and the correct amount of these factors is known to ensure the precise level of 70S ribosomes in the cell.
Ribonucleases are involved in maturation, degradation and quality control of RNA thus, assuring the optimal amount of each transcript in the cell. The RNB family is present in all domains of life and usually includes RNase R, RNase II and the eukaryotic Rrp44/Dis3, Dis3L1 and Dis3L2. While in *Escherichia coli* both RNase II and RNase R coexist, in *Streptococcus pneumoniae* only RNase R is present.
We explored the relevance of pneumococcal RNase R in translation by comparing the sucrose gradient polysome separation of the wild type with that of an RNase R mutant strain. We show that in the absence of RNase R the amount of the 70S ribosomal subunit decreases, while increasing the level of the free subunits. Expression studies of the ribosome dissociation factors shows that RNase R controls the level of these mRNAs. Accumulation of these factors in the mutant is most probably the cause for the decreased amount of 70S active particles, compromising translation and affecting cellular viability.
This investigation highlights the importance of RNase R in a fundamental cell metabolism process, which might thus be a promising candidate to use as target for antimicrobial treatment.

Keywords: Ribonuclease; translation; ribosome; degradation; protein synthesis.

Acknowledgements*: This work was financially funded by Fundação para a Ciência e a Tecnologia (BD/99477/2014).*

O7. Seed development in *Phaseolus vulgaris* L.: post-transcriptional regulation mediated by miRNAs

[1]*Parreira, J.R., [2]Cappuccio, M., [2]Balestrazzi, A., [1,3]Fevereiro, P. & [1,2]Araújo, S.S.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras 2780-157, Portugal*
[2]*University of Pavia, Pavia 27100, Italy*
[3]*Faculdade de Ciências da Universidade de Lisboa, Lisboa 1749-016, Portugal*
lead presenter: jsalvado@itqb.unl.pt

Seed development (SD) is one of the most relevant developmental processes in grain legumes since it determines seed storage composition, which impacts seed traits like yield and nutritional value. MicroRNAs (miRNAs) are endogenous non-coding small RNAs (~21-nt) that act as post-transcriptional regulators of gene expression. The knowledge of miRNAs that act during SD is still limited, namely in the grain legume *Phaseolus vulgaris*.
Our objective is to identify miRNAs that act during SD and their respective targets, and understand how the timely accumulation of miRNAs can regulate gene expression. For this purpose the dynamics of miRNAs expression (sRNA-Seq) were analyzed in 4 times points during SD, spanning late embryogenesis to seed desiccation. Furthermore, target prediction analysis and degradome sequencing was also performed.
Expression of 594 putative miRNAs was observed during SD. Of those, 69 were previously described and 42 were selected as new candidates. As expected, most changes in miRNA expression were found at the initial time points. Target prediction analysis revealed transcripts that encode several subunits of the NUCLEAR FACTOR Y (NF-Y) transcription factor as putative targets of MIR169 gene family members. One of those NF-Y subunits is the *NUCLEAR FACTOR Y, SUBUNIT A3* (*NF-YA3*) that was previously shown to be required for embryo development in *Arabidopsis*. In another case, the degradome analysis showed that a putative candidate miRNA targets the *STRESS INDUCED PROTEIN* (*EM1*), a *LATE EMBRYOGENESIS ABUNDANT* gene, which may have a role in seed desiccation.
The qPCR expression profiles of selected miRNAs is underway, aiming to validate the sRNA-Seq analysis. In the long run, this knowledge will be concatenated with the transcriptome and proteome data to have a comprehensive view of the molecular factors modulating common bean seed development.

Keywords: Seed development; *Phaseolus vulgaris*; miRNA.

Acknowledgements*: This work was financially funded by Fundação para a Ciência e a Tecnologia (BD/113474/2015).*

O8. Making translation right: Hfq is a novel ribosome biogenesis factor

*,[1]dos Santos, R.F., [1]Andrade, J.M. & [1]Arraiano, C.M.

[1] *Instituto de Tecnologia Química e Biológica (ITQB NOVA), Universidade Nova de Lisboa, Oeiras, Portugal*
**lead presenter: ricardo.santos@itqb.unl.pt*

The ribosome is the macromolecular machine responsible for translation. Defects during ribosome production ultimately lead to dysfunctional particles that will endanger protein synthesis. The assembly of ribosomes relies on ribosome biogenesis factors that guide the correct folding and binding of the ribosomal RNA and ribosomal proteins.
In this work we assigned novel roles in the critical processes of ribosome biogenesis and rRNA quality-control for the RNA-binding protein Hfq. This RNA chaperone is commonly known to facilitate sRNA-mRNA basepairing. Here, we show for the first time that Hfq selectively binds immature 30S subunits which holds misprocessed 16S rRNA (termed 17S rRNA). We have found that Hfq binds to 16S rRNA and is critical for its correct folding. Strikingly, inactivation of Hfq leads to decreased levels of the fully functional 70S ribosomes. This has severe consequences for cell translation, resulting in a global reduction in protein synthesis as revealed by Ribo-seq. Accordingly, in a Δ*hfq* strain the protein levels of translation related genes are lowered, whilst mRNA levels remain generally unchanged. Hfq depletion also results in increasing miscoding events further highlighting its importance for translation fidelity. Moreover, the distal face of Hfq is specifically required for ribosome regulation, showing this function is independent of Hfq role in sRNA biology. Further work revealed that Hfq can interact with RNase R, in a novel rRNA quality-control pathway.
In summary, we have demonstrated that the extensively studied Hfq is a new ribosome assembly factor. Cells lacking Hfq exhibit diverse hallmarks of ribosome biogenesis defects, namely: i) misprocessing and accumulation of 17S rRNA; ii) reduced pool of 70S ribosomes; iii) abnormal translation and compromised translation fidelity. This work expands the functions of Hfq beyond the small non-coding RNA biology and unveils unprecedented roles in ribosome biogenesis and rRNA quality-control.

Keywords: Hfq; Quality-control; Ribosome biogenesis; rRNA; translation.

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O9. A motif similar to the GCC-box directs bundle sheath-specific expression of the gene encoding the maize C_4 NADP-ME enzyme

[1,2]*Borba, A.R., [3]Reyna-Llorens, I., [3]Kneřová, J., [3]Wang, N., [3]Stanley, S., [1,2]Saibo, N. J. M. & [3]Hibberd, J.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157, Oeiras, Portugal*
[2]*Instituto de Biologia Experimental e Tecnológica, 2780-157, Oeiras, Portugal*
[3]*Department of Plant Sciences, Downing Street, University of Cambridge, Cambridge, CB2 3EA, UK*
**lead presenter: borba@itqb.unl.pt*

C_4 photosynthesis is a complex phenotype that allows more efficient carbon capture compared with the ancestral C_3 cycle, accounting for approximately 25% of primary productivity on the planet. Strict compartmentation of carbon dioxide (CO_2) assimilatory enzymes between mesophyll (M) and bundle sheath (BS) cells is a hallmark of the C_4 pathway. However, the regulatory mechanisms underlying restriction of gene expression to these two distinct photosynthetic cell-types are poorly understood. Here, we studied the spatial patterning of gene expression of *C_4-NADP-dependent Malic Enzyme* (*NADP-ME*) from *Zea mays* in C_3 *Oryza sativa* and C_4 *Gynandropsis gynandra*. Transgenic reporter assays showed that a 235 base pair promoter region of the *ZmC_4-NADP-ME* gene confers BS-specificity in C_4 but despite being recognised by C_3 *trans*-factors it is not actively functional in C_3 leaves. This BS-specificity in *G. gynandra* (dicot) directed by a *Z. mays* (monocot) sequence indicates that *cis*-elements involved in the regulation of the *NADP-ME* gene were co-opted throughout C_4 evolution and their cognate *trans*-factors have conserved properties in both C_4 plant lineages. To identify *cis*-regulatory motifs within the promoter of *ZmC_4-NADP-ME* that are responsible for BS-preferential expression we combined functional and computational analyses. A six base pair GCC box-like motif was found to act as a positive regulator of BS-specificity in C_4 leaves. This BS activator motif could then be used to engineer C_4-like gene expression in the BS of C_3 plants.

Keywords: Photosynthesis; NADP-dependent Malic Enzyme; GCC box-like *cis*-element; cell-specificity.

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O10. Specialisation events of fungal metacommunities exposed to a persistent organic pollutant are suggestive of augmented pathogenic potential

[1]*Martins, C., [1,2]Varela, A., [3] Leclercq, C.C., [4]Núñez, O., [5]Větrovský, T., [3]Renaut, J., [5]Baldrian, P. & [1,7]Silva Pereira, C.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157, Oeiras, Portugal
[2]Instituto Nacional Investigação Agrária e Veterinária, Av. da República, 2780-157, Oeiras, Portugal
[3]Integrative biology platform, Environmental Research and Technology Platform, Luxembourg Institute of Science and Technology, Belvaux, Luxembourg
[4]Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain
[5]Serra Hunter Fellow, Generalitat de Catalunya, Spain
[6]Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, 14220 Praha 4, Czech Republic
[7]University of the West of Scotland, Paisley Campus, PA1 2BE Paisley, UK
**lead presenter: celso@itqb.unl.pt*

The impacts of man-made chemicals, in particular of persistent organic pollutants, are multifactorial as they may affect the integrity of ecosystems, alter biodiversity and have undesirable effects on many organisms. We have demonstrated that Pentachlorophenol (PCP), an archetypal Persistent Organic Pollutant (POP), is contaminating remote Tunisian cork oak forest soils and that the belowground mycobiota of forest soils acts as a buffer against PCP. However the trade-offs made by mycobiota to mitigate this pollutant remained cryptic. Herein we demonstrate using a culture-dependent approach that exposure to pentachlorophenol led to alterations in the composition and functioning of the metacommunity, many of which were not fully alleviated when most of the biocide was degraded. Proteomic and physiological analyses showed that the carbon and nitrogen metabolisms were particularly affected. This dysregulation is possibly linked to the higher pathogenic potential of the metacommunity following exposure to the biocide, supported by the secretion of proteins that have been found to be associated pathogenicity and reduced susceptibility to a fungicide. Our findings provide additional evidence for the silent risks of environmental pollution, particularly as it may favour the development of pathogenic trade-offs in fungi, which may impose serious threats to animals and plant hosts. The stimulation of increased fungal pathogenicity due to POP exposure is not something that can be ignored.

Keywords: Pentachlorophenol; fungal metacommunities; amplicon sequencing; metabolic dysregulation; pathogenic potential.

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O11. Hydrophobic Deep Eutectic Solvents: a new approach to Water Cleaning

[1]*Florindo, C., [2]Branco, L.C. & [1,3]Marrucho, I.M.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. Da República, 2780-157, Oeiras, Portugal
[2]REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, 2829-516 Caparica, Portugal
[3]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais, 1049-001 Lisboa, Portugal
**lead presenter: catarina.florindo@itqb.unl.pt*

The development of highly sophisticated analytical techniques allowed to detect the presence of micropollutants in aquatic ecosystems, which has related to major public health problems. A list of priority compounds, that must be urgently removed from water sources, has been issued and water innovative solutions are being proposed at a fast pace. Among the priority compounds, pharmaceuticals play a relevant role, due to their large consumption intimately related to human well-being. This work addresses the removal of pharmaceutical micropollutants from several classes, endocrine disruptors, non-steroid anti-inflammatory drugs and antibiotics from aquatic environments. Liquid-liquid extraction is an efficient and promising technique, which can be easily scaled up and operated at low costs. The major challenge in liquid-liquid extraction lays in the choice of an appropriate solvent, which is effective and environmentally friendly. This work focuses on the development and characterization of hydrophobic deep eutectic solvents (DESs) as cheap extractants, using natural components, such as menthol, fatty acids and quaternary ammonium salts. Extraction efficiencies and the main factors affecting the extraction yield, such as time, stirring speed and molar ratio between DESs and water, were optimized as well as evaluation of the DES's ability to be recycled and reused. Moreover, and taking advantage of high surface/volume of extractant needed to implement an adsorption process, novel highly efficient technology based on hydrophobic DESs supported in a porous filter was successfully developed.

Keywords: Deep eutectic solvents; hydrophobicity; supported hydrophobic DESs; micropollutants; water cleaning.

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O12. Unraveling the molecular mechanisms of a successful graft in grapevine

[1*]Assunção, M., [2]Canas, S., [3]Eiras-Dias, J.E. &[4]Fevereiro, P.

[1]*Plant Cell Biotechnology Lab / Instituto de Tecnologia Química e Biológica António Xavier (Green-it Unit)-Universidade Nova de Lisboa, Av da Républica, Oeiras, Portugal; massuncao@itqb.unl.pt*
[2]*Unidade de Investigação de Viticultura e Enologia/ Instituto Nacional de Investigação Agrária e Veterinária (Biotechnology and Genetic Resources Unit), Quinta da Almoinha, Dois Portos, Portugal and Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Instituto de Investigação e Formação Avançada, Universidade de Évora, Núcleo da Mitra, Évora, Portugal; canas.sara@itqb.unl.pt*
[3]*Unidade de Investigação de Viticultura e Enologia/ Instituto Nacional de Investigação Agrária e Veterinária (Biotechnology and Genetic Resources Unit), Quinta da Almoinha, Dois Portos, Portugal*;eiras.dias*@iniav.pt*
[4]*Plant Cell Biotechnology Lab / Instituto de Tecnologia Química e Biológica António Xavier (Green-it Unit)-Universidade Nova de Lisboa, Av da Républica, Oeiras, Portugal and* Departamento de Biologia Vegetal/Faculdade de Ciências da Universidade de *Lisboa, Campo Grande, Lisboa, Portugal*;
**lead presenter: maria.sm.assuncao@gmail.com*

Grafting is a very ancient technique of asexual propagation used to overcome biotic and abiotic stresses, to avoid juvenility and to improve quality. This technique is used worldwide in almost every fruit tree as well as in many horticultural crops. The use of grafting is mandatory in viticulture due to phylloxera, a small insect that feeds on roots and leave vines to death. The only way to overcome this pest is by grafting the cultivars of interests in American or hybrid resistant rootstocks. Despite its economic importance, the lack of graft success is still a widespread problem and the molecular mechanisms of graft union are still poorly known. Graft success can be defined as the correct establishment of the vascular continuity between scion and rootstock, with survival and proper functioning of the new composite. Grafting success is a complex phenomenon that requires numerous biological events at the graft interface such as cell recognition and communication, initiation of cell cycle, proliferation and differentiation and plasmodesmata formation.
We are interested in understanding the molecular mechanism that drives a successful graft union in *Vitis* spp.
Two scion/rootstocks combinations with different graft success rates were compared by transcriptomics and metabolomics (phenolic compounds). Differences during graft union between the two systems were observed. An important balance between oxidative stress, signalling, and growth at the graft union seems to be crucial for the graft success and some phenolic compounds were found to be potential markers in graft success prediction. Furthermore, two transcription factors were chosen for functional analysis with the aim of contributing to decipher the regulatory network(s) involved in graft union success.

Keywords: *Vitis* spp., graft success, transcriptomics, phenolic compounds, functional analysis

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O13. Growth and physiology of maize seedlings respond differently to variations in water content and irrigation method

[1,2,3*]Rodrigues, J., [1,2]Wuyts, N., [1,2]Demuynck, K., [1.2]Nelissen, H., [3]Saibo, N. & [1,2]Inzé, D.

[1]*Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium*
[2]*VIB Center for Plant Systems Biology, 9052 Ghent, Belgium*
[3]*Instituto de Tecnologia Química e Biológica António Xavier, UNL, 2780-157 Oeiras, Portugal*
**lead presenter: joana.rar@itqb.unl.pt*

Drought leads to tremendous crop yield losses and is predicted to become more frequent and severe. Preservation of plant growth and photosynthesis are the traits most associated with improved crop yield under drought, though the mechanisms driving growth and photosynthesis responses to drought are still debatable. For decades, the reduction of plant growth upon drought was thought to be a consequence of carbon starvation due to reduced photoassimilation. However, it has been shown that a severe growth reduction in response to drought can take place before significant photosynthesis impairment. To reveal the molecular mechanisms driving growth and photosynthesis responses to drought, we developed an experimental design where plants reduce either growth or photosynthesis. Decreasing the amount of applied water leads to photosynthesis impairment, reduced root growth but no significant reduction in leaf growth. Contrastingly, changing the irrigation method did not affect photosynthesis, but altered root architecture and reduced leaf growth due to a decrease in the number of dividing cells, indicating that growth reduction was due to a lower cell division rate instead of a reduced cell expansion. Measurements of soil and leaf water content showed that the amount of applied water, which is typically used as indicator for mild or severe drought, did not correspond to the amount of water available to the plant. These results were obtained in two different laboratories and the growth reduction with no alteration in photosynthesis was also obtained using an automated phenotyping platform, showing the robustness of our observations. Currently, we are performing RNAseq analysis to identify the transcriptional changes underlying the distinct responses observed. This knowledge will aid understanding the relationship between growth and photosynthesis under drought, and identify pathways that can be targeted to improve crop drought tolerance.

Keywords: drought; phenotyping; growth rate; photosynthesis; water method.

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O14. Combined plant abiotic stress: the impact of salt and heat in the model actinorhizal plant *Casuarina glauca*

[1]*Jorge, T.F., [2,3]Ramalho, J.C., [4]Pais, I.P., [2,3]Leitão, A.E., [2]Rodrigues, A.P., [2,3]Leitão, A.E., [5]Alseekh, S., [3,4]Scotti-Campos, P., [1,2,3]Ribeiro-Barros, A.I., [5]Fernie, A.R. & [1]António, C.

[1]*Plant Metabolomics Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa (ITQB NOVA), Av. da República, Oeiras, Portugal;*
[2]*PlantStress&Biodiversity Lab, Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa & Oeiras, Portugal;*
[3]*GeoBioTec, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal;*
[4]*Unid. Investigação em Biotecnologia e Recursos Genéticos, Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV), Oeiras, Portugal;*
[5]*Central Metabolism Group, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany.*
*lead presenter: *tiagoj@itqb.unl.pt*

Actinorhizal plants have been regarded as promising species in the current climate change context, due to their ability to tolerate harsh environmental conditions. Previous studies have reported that the model actinorhizal plant *Casuarina glauca* Sieb. ex Spreng could withstand increasing salt concentrations by maintaining a controlled oxidative environment inside the cells, and performing osmotic adjustments that contribute to maintain a relevant integrity of the photosynthetic machinery [1-2]. Through a mass spectrometry (MS)-based metabolomics approach, we reported that modifications in the levels of neutral sugars, proline and ornithine were associated with *C. glauca* salt stress tolerance [3] and that a flavonoid secondary antioxidant complements the ascorbate-glutathione cycle components, thereby supporting the maintenance of the stable oxidative environment within the plant cells [4].
However, under natural environmental conditions, plants are routinely exposed to a combination of different stresses, a situation that is largely exacerbated in a context of climate changes. Several studies have been reporting that plant responses to combined stresses should not be regarded as a sum of the responses from each individual stress. The interaction of salt and heat stresses has shown both positive and negative impacts in plant physiology [5]. That said, the present study aims at investigating *C. glauca* physiological and metabolic responses to combined salt and heat stresses exposure, thus, contributing for a better understanding of the impact of environmental stresses in this ecologically significant species. In this oral presentation, our most recent results on a MS-based plant metabolomics approach (GC-TOF-MS primary metabolite profiling and LC-MS/MS secondary metabolite profiling) coupled to *C. glauca* physiological measurements will be discussed.

Keywords: Actinorhizal plants; Metabolomics; Combined stresses; Salt stress; Heat stress.

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O15. Two novel *Arabidopsis thaliana* membrane transporters regulate plant growth and development in response to environmental signals

[1,2]*Niño-González, M. & [2]Duque, P.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
[2]*Instituto Gulbenkian de Ciência, 2780-156, Oeiras, Portugal*
lead presenter: mfgonzalez@igc.gulbenkian.pt

The Major Facilitator Superfamily (MFS) of membrane transporters remains barely studied despite being ubiquitous in living organisms and representing the second largest group of transporters on Earth. Our lab's previous work on the functional characterization of plant MFS transporters has revealed not only key roles in the response to environmental cues, but also striking examples of the biological relevance of alternative splicing in plant systems. My functional analysis of *Arabidopsis thaliana* genes encoding uncharacterized members of the MFS has uncovered two novel transporters, which we provisionally named MFS12 and MFS18, involved in the plant´s response to abiotic stress. Loss-of-function mutants for *MFS12* are hypersensitive to cadmium (Cd) and arsenic (As) when subjected to iron (Fe) deprivation. Fe deficiency alone, or Cd or As treatment in Fe-sufficient conditions, affects equally mutant and wild-type roots, suggesting a role for MFS12 in metal detoxification specifically when environmental conditions, such as Fe deprivation, enhance the likelihood of metal uptake. I found that the *MFS12* gene generates five different mRNAs, two of which give rise to the full-length protein. Interestingly, stressed roots express only these two transcripts. On the other hand, mutants for *MFS18* exhibit hypersensitivity to chilling (4-9°C) during seed germination and elongation of the hypocotyl, indicating that MFS18 modulates the response to cold during the early stages of plant development. Like *MFS12*, the *MFS18* gene also gives rise to different transcripts generated via alternative splicing/polyadenylation, whose regulation under cold conditions is currently being investigated. With these studies, together with ongoing complementation, subcellular localization and promoter-reporter analyses, as well as transport assays in yeast, I aim at unveiling the molecular link between the observed stress phenotypes and the function of the MFS12 and MFS18 plant membrane transporters.

Keywords: *Arabidopsis thaliana*; MFS transporter; chilling stress; heavy metal stress; iron deficiency.

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O16. How far do we need to go back in time to recover the representative genetic diversity in an olive tree cultivar? The "Galega" case study

[1,2]*Sales, H., [1]Alves, M.L., [2]Nunes, J. & [1]Vaz Patto, M.C.

[1]ITQB NOVA-Instituto de Tecnologia Química e Biológica António Xavier Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal
[2]BLC3 Association - Technology and Innovation Campus, Rua Nossa Senhora da Conceição, 2, Lagares, 3405-155, Oliveira do Hospital, Portugal
**lead presenter: helia.sales@itqb.unl.pt*

The cultivated olive tree (*Olea europaea* L. subsp. *europaea*) is a perennial plant widely cultivated throughout the world and clonally propagated for centuries. In Portugal, the main variety with economic importance, "Galega", has been replaced by more productive foreign varieties, leading to an intense reduction on its cultivation. This potential genetic erosion may be endangering the survival of "Galega". In this context, the characterization of the existing variability in this national variety becomes a priority and will contribute to the selection of the most interesting genotypes to ensure its future efficient use and conservation.
To tackle this problem, we used 14 microsatellite molecular markers to evaluate the genetic relationships among 341 accessions of centenary "Galega" olive trees, that represent the germplasm still in production at the Portuguese orchards. We observed a low intra-variety genetic variability, as given by the values of the several genetic diversity parameters such as expected heterozygosity, and the genetic distance between accessions, with no genetic differentiation detected between orchards. Still, we identified a total of 88 different alleles.
To unravel if this current low intra-variety genetic variability was due to a recent genetic erosion event, we performed a new prospection in the national territory and, characterized molecularly more 264 ancient "Galega" olive trees (500-2000 years old). In this new set of ancient "Galegas", we identified a total of 87 different alleles. Importantly, we observed no significant differences in the genetic diversity between the centenary and ancient olive trees. Similarly to what was observed in centenary "Galegas", low levels of genetic intra-variability were also found in ancient "Galegas".
With this study, novel insights on olive genetic diversity in "Galega" variety were presented.
Despite the low intra-variability found, we were able to genetically discern unique "Galegas" accessions, which is very important, since they reflect the variability that we need to preserve and will allow future genetic studies for the development of essential molecular tools to improve the variety.

Keywords: *Olea europaeae*; genetic diversity; SSRs; ancient olive trees; centenary olive trees.

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O17. Molecular mechanisms underlying peridermis suberization in Arabidopsis and Cork

[1,2]*Leal, A.R., [1]Barros, P., [1]Belo, J., [2]Beeckman, T. & [1]Oliveira, M.M.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA) & iBET, GPlantS, Av. da República, 2780-157 Oeiras, Portugal*
[2]*Department of Plant Systems Biology, VIB, Gent, Belgium & Department of Plant Biotechnology and Bioinformatics, Gent University, Gent, Belgium*
lead presenter: ana.leal@itqb.unl.pt

Land plants have developed specialized lipid and phenolic layers as protective barriers, both at root and shoot level. Suberin is one of these constituents, which is deposited on the cell wall, at both endodermis (tissue-tissue interface) and peridermis (plant-environment interface). Suberization of peridermis cells is a tightly controlled process regulated by endogenous and exogenous factors, however its development and regulatory mechanisms are still poorly understood. For a better understanding of the suberization process in the *Arabidopsis thaliana* root peridermis, we have performed a detailed chronological study analysing anatomically the activation of specific genes involved in the suberin biosynthetic pathway (*FAR4* and *GPAT5*) and suberin deposition (using specific stainings). We demonstrated that first cells undergoing peridermis differentiation were visible at 8 days after germination, with specific activation of suberin biosynthesis genes in pericycle daughter cells, being followed by suberin deposition. During the subsequent days, periclycle divisions for peridermis tissue are gradually intensified and more cells undergo suberization. Furthermore, we observed that the combination of osmotic and heat stresses at this developmental stage lead to strong modifications on suberization patterns, highlighting the dynamic nature of suberization upon stress exposure, particularly after the onset of root secondary growth. To identify tissue-specific molecular regulators during peridermis differentiation, a transcriptomic experiment targeting the suberizing cells is ongoing, using TRAP-SEQ technology[1] .
Furthermore, this study was extended to cork oak (*Quercus suber*) roots, where the application of a combination of heat and drought also affected peridermis development and suberization. By integrating the results from both systems we will deepen our knowledge on the impact of abiotic stress in cork development.

Keywords: Suberin; Peridermis; Root development; Drought; Cork.

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O18. The *atroviolacea* gene encodes a R3-MYB protein repressing Anthocyanin synthesis in tomato plants

[1]*Colanero, S., [1]Perata, P. & [1]Gonzali, S.

[1] *PlantLab, Institute of Life Sciences, Scuola Superiore Sant'Anna, San Giuliano Terme (Pisa), Italy*
**lead presenter: s.colanero@santannapisa.it*

The anthocyanin biosynthetic pathway is well characterized in plants. However, in tomato (*Solanum lycopersicum* L.) an exhaustive knowledge of its regulation is still lacking. Tomato mutants showing higher levels of anthocyanins in fruits or vegetative tissues, such as *Anthocyanin fruit (Aft*) or *atroviolacea* (*atv*), have been extensively exploited in the attempt to clarify the process. Nevertheless, only candidate genes have been proposed as responsible for such phenotypes. The recessive *atv* mutation likely represents an allelic variant of a gene introgressed in tomato from wild Solanum species. We performed genome sequencing of *atv/atv* plants followed by candidate gene analysis, and identified a mutated gene encoding an R3-MYB protein. When overexpressed, this protein abolished anthocyanin production in tomato seedlings and plants, by silencing key regulators and biosynthetic genes of the pathway. The functional analysis of the protein clearly showed that it can negatively interfere with the activation of the anthocyanin biosynthetic pathway mediated by the endogenous MYB-bHLH-WDR (MBW) complexes. In particular, this R3-MYB protein can directly bind the bHLH factors which are part of the MBW complexes, therefore acting as a competitive inhibitor. The R3-MYB protein here described is therefore involved in a feedback mechanism that dampens the production of anthocyanins once activated by endogenous or exogenous stimuli. The *atv* mutation causes the production of a truncated version of the R3-MYB factor that cannot retain the full potential to inhibit the MBW complexes, thus leading to a constitutively higher production of anthocyanins.

Keywords: Anthocyanin; *atv*; MBW complex; R3-MYB; tomato.

O19. Structural and Functional Studies on *Staphylococcus aureus* Peptidoglycan Amidation

[1]*Leisico, F., [1]Silva, M., [2,3]Figueiredo, T.A., [4]Morlot, C., [4]Vernet, T., [4]Zapun, A., [1]Cabrita, E.J., [2]Sobral, R.G., [2]Ludovice, A.M., [1]Romão, M.J., [3,5]de Lencastre, H. & [1]Santos-Silva, T.

[1]UCIBIO, Department of Chemistry, Faculty of Science and Technology, Universidade Nova de Lisboa; Caparica, Portugal;
[2]UCIBIO, Department of Life Sciences, Faculty of Science and Technology, Universidade Nova de Lisboa; Caparica, Portugal;
[3]Laboratory of Molecular Genetics, Microbiology of Human Pathogens Unit, Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa; Oeiras, Portugal;
[4]Université Grenoble Alpes, CNRS; CEA, IBS UMR 5075, 38044 Grenoble, France;
[5]Laboratory of Microbiology and Infectous Diseases, The Rockefeller University; New York, USA
**lead presenter: franciscoleisico@fct.unl.pt*

Peptidoglycan amidation is a key structural modification of the bacterial cell wall and it has been associated with antibiotic resistance mechanisms in Gram-positive bacteria [1]. The amidation reaction is carried out by the bi-enzymatic complex MurT-GatD in a two-step reaction: the glutaminase GatD produces ammonia from glutamine that is then transferred to MurT for the second reaction, where the peptidoglycan precursor lipid II is amidated [2,3]. In this work, we showed the functional determinants for the glutaminase reaction of MurT-GatD, combining the first crystal structure of GatD [4,5], from *Staphylococcus aureus*, and activity studies performed by ^{1}H-NMR spectroscopy [5]. The protein complex presented glutaminase activity even in the absence of lipid II and the mutants R128A, C94A and H189A were totally inactive, revealing their essential role in glutamine sequestration and its deamidation reaction. Recently, we got 3 Å diffraction data for the Murt-GatD complex, which we combined with SAXS data and kinetics to get the protein dynamics and functional determinants within the reaction mechanism of peptidoglycan amidation. All the multi-complementary data is integrated to open the landscape, both structural and functionally, on MurT-GatD complex to further develop structure-based drug design approaches. Given the ubiquitous presence of MurT-GatD in Gram-positive bacteria, especially in pathogenic species as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, these results reveal significant insights into the molecular basis of the so far undisclosed amidation mechanism, contributing to the development of alternative therapeutics to fight bacterial infections.

Keywords: *Staphylococcus aureus*; Peptidoglycan amidation; Murt-GatD protein complex; Structural Biology; X-ray Crystallography.

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O20. Computational studies on the function of ABC transporters

[1*]Abreu, B., [1,2]Oliveira, A. S. F., [1]Soares, C.M.

[1]*ITQB-NOVA, Universidade Nova de Lisboa, Av. da República 2780-157, Oeiras, Portugal*
[2]*School of Biochemistry & Center for Computational Chemistry, University of Bristol, University Walk, BS8 1TDS, Bristol, UK*
lead presenter: barbara.abreu@itqb.unl.pt

ATP binding cassette transporters (ABC) are transmembrane proteins that use energy from ATP hydrolysis for harnessing substrate translocation, undergoing substantial conformational changes [1]. They can be either importers or exporters. ABC transporters can play a role in multiple phenomena such as nutrient intake, regulation, drug resistance and even human diseases such as cystic fibrosis and sitosterolemia, to mention a few examples[1] [2].
Although these proteins possess a common structure with two nucleotide binding domains and two transmembrane domains, there are mechanistic differences between transporters[3]. Furthermore, recent evidence suggests new roles for ABC transporters that go beyond the traditional cycle of substrate transport and ATP hydrolysis[4].
In this work, we will report on several open questions that concern the function and mechanism of different ABC transporters that have biological relevance and that are model systems in the ABC transporter class.

Keywords: Molecular dynamics simulation; membrane proteins.

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O21. Functional and structural characterization of Alternative Complex III

[1*]Calisto, F., [2]Sousa, J.S., [2]Mills, D.J., [3]Langer, J.D., [1]Refojo, P.N., [1]Teixeira, M., [2]Kühlbrandt, W., [2]Vonck, J. & [1,4]Pereira, M.M.

[1]Instituto de Tecnologia Química e Biológica – António Xavier, Universidade Nova de Lisboa, ITQB NOVA, Oeiras, Portugal;
[2]Department of Structural Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany;
[3]Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany;
[4]Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
**lead presenter: fcalisto@itqb.unl.pt*

The electron transfer through the membrane complexes of the respiratory chain is coupled to the generation of the proton-motive force that drives the synthesis of ATP. Complexes I and IV couple electron transfer with transport of protons across the membrane, while complex III, the bc_1 contributes to the proton gradient by a Q-cycle mechanism.
Alternative Complex III (ACIII) is a quinol:cytochrome *c*/HiPIP oxidoreductase membrane complex widespread in the Bacteria domain, and a functional substitute of the bc_1 [1,2].
The structure of ACIII from *Rhodothermus marinus*, recently solved by cryo-electron microscopy at 3.9 Å resolution [3], shows three integral transmembrane subunits and four periplasmic subunits. The periplasmic domain accommodates six hemes and four FeS clusters, which form two divergent electron transfer wires. The two putative proton pathways and the quinol-binding site identified in ACIII structure suggest that ACIII operates by a redox-driven proton translocation mechanism, totally unrelated to the Q-cycle of complex III.
We aim to functionally characterize ACIII and its potential electron shuttles, for which we used several complementary biochemical and biophysical approaches including enzymatic assays, fluorescence spectroscopy and isothermal titration calorimetry.

Keywords: Respiratory chain; energy transduction; proton pump.

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O22. Functional and structural characterization of human microbiome systems for glycan recogniton in the gut

[1]*Correia, V.G., [1]Costa, R.L., [1]Trovão, F., [1]Pinheiro, B.A., [2]Brás, J.L.A., [3]Liu, Y., [3]Silva, L.M., [1]Romão, M.J., [3]Chai, W., [3]Feizi, T., [2,4]Fontes, C.M.G.A., [1]Carvalho, A.L. & [1]Palma, A.S.

[1]*UCIBIO, Department of Chemistry, FCT-NOVA, 2829-516 Caparica, Portugal*
[2]*NZYTech, Lda - Genes & Enzymes, 1649-038 Lisboa, Portugal*
[3]*Glycosciences Laboratory, Department of Medicine, FM-ICL, W12 0NN London, United Kingdom*
[4]*CIISA, FMV-ULisboa, 1300-477 Lisboa, Portugal*
**lead presenter: vivianacorreia@campus.fct.unl.pt*

From meal to meal, the human gut is flooded with a great diversity of glycans (carbohydrates) derived from diet or from host-cell mucus-layer. The microbial community living there – the microbiome, has evolved to readily target and utilize the different glycans as nutrients, influencing host nutrition, immunity and health [1].
Prominent microbiome strains, such as *Bacteroides spp*., exhibit extensive sets of substrate-specific genes that allow bacteria to cope with nutrient fluctuation [1]. Each set encodes all the necessary elements for the recognition and degradation of a specific glycan, including carbohydrate-active enzymes (CAZymes) associated with carbohydrate-binding modules (CBMs), that may reflect the systems specificity [2]. In the genomic Era, there is an urgent need to apply high-throughput (HTP) approaches to study these recognition systems at a functional and structural level.
In this communication, we walk you through our integrative strategy to characterize novel human microbiome glycan-recognition systems, combining HTP protein production of over 100 different putative glycan binding domains, ligand discovery using glycan microarray technology [3] and structural characterization of new protein-ligand complexes with X-ray crystallography [4]. Alternative glycan microarrays were used to cover, in a miniaturized format, the glycan structural diversity found in the gut including mammalian-, plant- or pathogen-derived glycans.
We highlight our findings on: 1) a newly identified protein from *B. ovatus* involved in the degradation of mixed-liked β-glucans, a type of dietary glycans with immunomodulatory effect; And 2) a system from *B. thetaiotaomicron*, capable of recognizing host-mucins derived sequences and milk oligosaccharides.
In future, this strategy can be extended to other microbial strains to help unravel the holistic effect of glycan recognition in the gut and pinpoint target pre- and probiotics for novel biopharmaceutical solutions.

Keywords: Human Microbiome; Protein-Glycan (Carbohydrate) interactions; Glycan Microarrays; X-ray crystallography.

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O23. Exploring the conformational and structural properties of the influenza fusion peptide in membrane bilayers: a computational study

[1]*Cruz, D.R., [1]Lousa, D. & [1]Soares, C.M.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*
lead presenter: davide@itqb.unl.pt

One the key players involved in the entry of the influenza virus in the host cells is the hemagglutinin protein. After the viral uptake by endocytosis and consequent lowering of the pH, this protein suffers major conformational changes. This enables the fusion of the viral envelope and host cell membrane, allowing the entry of the genetic material that will resume the infection process [1]. One particular region of the hemagglutinin protein, called the fusion peptide (FP), has a major role in the membrane fusion process. However, the molecular determinants behind the action of the FP in this process are yet to be elucidated, and experimental studies have not been able to determine the conformation of the FP inside the membrane. A study by our group has brought new insights on this subject [2]. In the present work, we expand our knowledge on the conformational and structural properties of the FP in a model membrane using enhanced sampling computational methods, such as well-tempered metadynamics simulations.
The results of this work will contribute with a better understanding of the mechanisms of membrane fusion that occur during influenza virus infection.

Keywords: Metadynamics; fusion; influenza; hemagglutinin.

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O24. Improving O_2-stability in a [NiFeSe] hydrogenase

[1]*Zacarias, S., [1]Temporão, A., [2]Del Bario, M., [2]Fourmond, V., [2]Léger, C., [1]Pereira, I. A. C. & [1,3]Matias, P.M.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal
[2]Aix-Marseille Université, CNRS, BIP UMR 7281, Institut de Microbiologie de la Méditerranée, 31 Chemin J. Aiguier, 13402 Marseille Cedex 20, France
[3]Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
**lead presenter: sonia.zacarias@itqb.unl.pt*

Hydrogenases are enzymes that efficiently catalyze the production and oxidation of hydrogen ($H_2 \rightleftharpoons 2H^+ + 2e^-$), having the potential to be used as biocatalysts in a future "hydrogen economy". However, the most catalytically active hydrogenases are sensitive to O_2, hindering their use as biotechnological tools.
The [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* Hildenborough (*Dv*H) has high H_2 production (≈8000 s^{-1}) and oxidation (≈4500 s^{-1}) activities and low product inhibition[1]. It has already been shown to be a good catalyst in photocatalytic H_2 production[2] and in H_2 oxidation in a biofuel cell[3].
This enzyme is routinely purified and crystallized under air and develops an irreversible oxidation of a Ni-coordinating active-site cysteine (Cys75) to sulfinate. It is not well understood how this oxidation occurs, since this residue is not directly reachable via a hydrophobic channel through which small diatomic molecules like O_2 reach the active site. Also, this type of oxidation was never observed for a [NiFe] hydrogenase.
We created several variants of the *Dv*H [NiFeSe] hydrogenase, with mutations in a residue at the end of a solvent channel that leads to Cys75. Two variants succeed in preventing Cys75 oxidation, as revealed by their X-ray crystal structure. Additionally, these two variants are less inhibited by O_2 than the wild-type and can be reactivated to their maximal activity even after long-term exposure to air. So, the two variants of the [NiFeSe] hydrogenase are more resistance to O_2 without compromising the high catalytic activities. These results also provide new insights on O_2 diffusion in solvent channels.

Keywords: Hydrogen; [NiFeSe] hydrogenase; *Desulfovibrio vulgaris*; catalytic activities; x-ray crystal structure.

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O25. Fluorinated Ionic Liquids for the Development of New Drug Delivery Systems

[1,2]*Vieira, N.S.M., [2]Araújo, J.M.M., [2]Rebelo, L.P.N. & [2]Pereiro, A.B.

[1]*Instituto de Tecnologia Química e Biológica António Xavier; Universidade Nova de Lisboa, Oeiras, Portugal;*
[2]*LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal;*
lead presenter: nicole.morgado@itqb.unl.pt

The design of new "greener" and sustainable pharmaceuticals is expected to integrate lower environmental harmfulness of both products and processes as one of the screening criteria in drug development. Therapeutic proteins make part of the most promising classes of biopharmaceuticals. However, there are some problems associated to the use of proteins, as their instability, short half-life in the bloodstream and the possibility to trigger immune responses [1]. To avoid these problems maintaining the therapeutic levels without side effects, the development of novel drug delivery systems (DDS) are being intensively studied.
Fluorinated ionic liquids (FILs) combine the best properties of both fluorinated surfactants (such as their chemical and biological inertness) with the greatest properties of ionic liquids (such as their high thermal stability and high tuneability) [2,3] which make them very attractive for the design of DDS.
The main purpose of this work is to evaluate the use of FILs as DDSs for therapeutic proteins. The initial screening studies were performed to select the most biocompatible and ecofriendly FILs. With this goal in mind, the cytotoxicity of FILs in four different human cell lines were performed. Their impact after being released in the aquatic environment, was evaluated through ecotoxicity tests performed in aquatic species with different levels of biological organization.
After the initial screening, different studies were performed using lysozyme, to examine the interactions and influence of FILs in therapeutic proteins. Activity and circular dichroism assays were performed to probe the chemical inertness of these ionic liquids for lysozyme. The encapsulation of the protein inside the structures formed by these FILs was supported by dynamic light scattering and microscopy studies. Finally, the encapsulation efficiency and the drug loading were also discussed.

Keywords: Sustainable pharmaceuticals; Drug development; Therapeutic proteins; Drug delivery systems; Fluorinated ionic liquids.

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O26. Chemo-enzymatic strategies for the sustainable exploitation of wasteful materials

[1]*Fernandes, A., [1]Bonardo, L., [2]Robalo, M. P., [1]Royo, B. & [1]Martins, L. O.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*
[2]*Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, IPL, Lisboa, Portugal/Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, UL, Lisboa, Portugal*
lead presenter: ana.fernandes@itqb.unl.pt

Enzymatic and organometallic catalysis offer complementary approaches for producing high-value chemicals through the application of new efficient and selective reactions. Their cooperative use can allow the valorisation of wastewater effluents, contributing to a circular economy with a smart and efficient use of resources. Azo dyes (-N=N-) and nitroaromatics (-NO_2) are widely used in different industries and represent largest groups of pollutants released to the environment. Enzymes identified and characterized with azoreductase and nitroreductase activity have shown a similar catalytic action producing the corresponding aromatic amines products [1]. These are important building blocks for a variety of industries, like agrochemical, fine chemical and pharmaceutical. These amines can be further valorised through enzymatic or chemical reactions, eliminating their intrinsic toxicity while simultaneously producing industrial relevant compounds, for example, through enzymatic oxidation reactions catalysed by a laccase or through N-alkylation with alcohols catalysed by organometallic complexes [2]. In order to convert azo-dyes into valuable compounds a highly efficient azoreductase from *Pseudomonas putida* MET94 (PpAzoR) [3] was used producing aromatic amines. Furthermore, the activity of PpAzoR in the conversion of nitroaromatics into amines was shown to result in promising preliminary results. Two methodologies were applied for the valorisation of these amines. In the first methodology, CotA-laccase was used as biocatalyst leading to the production of relevant heterocyclic compounds such as phenoxazinones, phenazines and quinones. The bioconversion of azo dyes was initially achieved using step-wise processes with purified enzymes and then processes using both free and immobilized recombinant *Escherichia coli* cells overproducing PpAzoR and CotA-laccase [4] were optimized. In the second methodology, a new water-soluble iridium (III) complex bearing N-heterocyclic carbene ligands was synthetized and its efficiency to catalyse the N-alkylation of different aromatic amines was demonstrated [5].

Keywords: Azo dyes; nitroaromatics; azoreductase; laccase; iridium NHC.

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O27. Recovery of bioactives from strawberry-tree fruit and its distillate residue using green technologies: a biorefinery approach

[1,2,3]*Alexandre, A.M.R.C., [1]Serra, A.T., [1]Matias, A.M., [3]Cocero, M.J. & [1,2,4]Bronze,M.R.

[1]*Instituto de Biologia Experimental e Tecnológica; Apartado 12, Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica António Xavier; Av. da República, Oeiras, Portugal*
[3] *High Pressure Processes Group, Universidad de Valladolid, Valladolid, Spain*
[4]*Faculdade de Farmácia da Universidade de Lisboa; Avenida Professor Gama Pinto, Lisboa, Portugal*
*lead presenter: *agostinho@ibet.pt*

Arbutus unedo is an evergreen plant from the Ericaceae family commonly known as the strawberry-tree fruit. Mediterranean endogenous and widespread in Portugal, its fermented fruits have been used for long to produce an alcoholic beverage known as "Aguardente de Medronho", after a distillation process. At final, the process produces a fermentation residue that figures as an environmental and economical issue for producers.

In this work, alternative extraction methods such as microwave technology, supercritical fluid extraction and pressurized water for the production of extracts rich in bioactive compounds were applied both to fresh fruit and to the residue. Results obtained are discussed and a comparison with conventional solid/liquid extractions is performed. These methods shown to be faster and present higher yields of recovery than conventional methods. Moreover need less solvents, which is in line with the principles of "Green Chemistry". Phytochemical composition of extracts was evaluated by different chromatographic methods as HPLC-DAD, LC-MS, and GC-MS. Bioactivity was tested for its antioxidant capacity by the ORAC method and for its antiproliferative activity towards cancerous HT-29 cell lines. Results shown that both fruit and the distillated solid waste stream still contains compounds of interest that can be used for the production of bioactive extracts with applications of fine chemistry.

Keywords: *Arbutus unedo*; Biorefineries; Extraction; Green Chemistry.

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O28. Manganese Organometallic Complexes in Catalysis

[1]*Pinto, M., [2]Franco, F., [2]Lloret-Fillol, J., [3]Albrecht, M. & [1]Royo, B.

[1] Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras (Portugal)
[2] Institute of Chemical Research of Catalonia (ICIQ), Avinguda Països Catalans 16, 43007 Tarragona (Spain)
[3] Departement für Chemie und Biochemie, Universität Bern, Freistrasse 3, CH-3012, Bern (Switzerland)
**lead presenter:mara.pinto@itqb.unl.pt*

In recent years, the development of catalysts based on abundant first-row transition metals has become a central topic in catalysis. Among 3d metals, manganese is particularly attractive for catalysis due to their natural abundance, and their unique features of being non-toxic and biocompatible. Thus, the last few years have witnessed a remarkable growth of interest in Mn-based catalysis. In this work, we describe the synthesis and characterisation of a new family of Mn organometallic complexes bearing N-heterocyclic carbene ligands and their application in catalysis, namely in the reduction of CO_2 and ketones, and in the oxidation of alcohols. We have disclosed the first purely organometallic complex with unprecedented activity for the selective electrocatalytic reduction of CO_2 to CO, displaying the highest TOF numbers (*ca*. 320000 s^{-1}) ever reported for a manganese-based catalyst.[1] In addition, we demonstrated the excellent catalytic activity of these complexes in the reduction of ketones to alcohols through hydrosilylation reactions.[2] Furthermore, we present the synthesis of unique examples of Mn complexes bearing triazolylidene ligands and their catalytic activity in the selective oxidation of alcohols to ketones.[3]

Keywords: Manganese; Catalysis; N-Neterocyclic carbenes.

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O29. Development of a novel strategy for protein purification through affinity magnetic precipitation

[1]*dos Santos, R., [1]Romão, M.J., [2]Goncalves, J., [1]Carvalho, A.L. & [1]Roque, A.C.A.

[1] *UCIBIO, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Portugal*
[2] *IMed- Research Institute of Medicines, Faculdade de Farmácia, Universidade de Lisboa, Portugal*
* *lead presenter: rar.santos@campus.fct.unl.pt*

Magnetic fishing using affinity-functionalized magnetic particles (MPs) is a proven method for target protein capture from complex mixtures [1]. Given the virtual infinite surface modifications that can be made on MPs' surface, we investigated the influence of MPs in protein crystal growth as nucleation agents. Functionalized and non-functionalized MPs were used as additives in lysozyme and trypsin crystallization. A rational design for MPs' functionalization was achieved, having MPs functionalized with chitin and casein for lysozyme and trypsin crystallization, respectively. The presence of functionalized MPs led to a faster crystal growth kinetics, still improving crystal yield without hampering crystal diffraction. The new magnetic crystallization method enables the possibility to overcome some protein crystallization difficulties, but also has the potential to be integrated in protein purification methods involving crystallization/precipitation steps. The new trend in downstream processing include Anything But Chromatography methodologies [2], namely precipitation [3] and magnetic fishing [1]. These two methods were used in synergy for antibody (Ab) purification. A screening of precipitation conditions in the presence of MPs functionalized with an affinity ligand for Ab were tested against human plasma and mammalian cells supernatant. Affinity driven magnetic precipitation enabled crude extract fractionation and Ab recovery in the elution fraction with high purity and recovery yield, up to 99% and 97%, respectively.

Keywords: Affinity; Protein crystallization; Protein precipitation; Protein purification; Affinity magnetic precipitation.

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O30. Streamlining Upstream Processing of Complex Biopharmaceuticals

[1,2*]Sousa, M., [1,2]Serra, M., [1,2]Marques, P., [1]Carrondo, M. & [1,2]Roldão, A.

[1]*iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
lead presenter: msousa@ibet.pt

Improving upstream processing of complex biopharmaceuticals using animal cell cultures is a continuous and multidisciplinary task that must be dealt case-by-case. Process intensification tools can assist the development of these processes, generating flexible and scalable production platforms. In this work, we evaluated the impact of (i) bioreactor design, (ii) perfusion, and (iii) scale-up/-down/-out on the yields and quality of two complex biopharmaceuticals: an oncolytic adenovirus vectors (Ad) produced in human lung carcinoma cells (A549) and a Peste des Petites Ruminants virus (PPRV) vaccine candidate produced in Vero cells.
The impact of new single-use bioreactor design (PBS Vertical-Wheel™ technology; PBS) on A549 cells growth and Ad production was assessed and compared to traditional stirred-tank bioreactor (STB). Process transfer and scale-up/-out from STB to PBS was successfully carried out and results show that cell growth rate and infectious virus concentration and quality (ratio of total to infection virus) could be improved in the PBS.
Perfusion was used to develop a new PPRV vaccine production process. Growing Vero cells adapted to serum-free medium on microcarriers in 2L STB under perfusion enabled a two-fold increase in maximum cell concentration when compared with batch culture. Moreover, these cells were detached from microcarriers using a new *in-situ* detachment protocol with cell recovery yields above 80%. Together, these methods enabled the scale-up to 20L STB directly from a 2L STB, surpassing the need for a mid-scale platform and reducing seed-train preparation time. Continuous depth filtration for PPRV clarification was successfully carried out with yield higher than 90%. Process scalability was validated at the 20L scale by comparing cell growth kinetics and metabolism as well as PPRV production kinetics to those achieved in 2L STB.

Keywords: Single-use; perfusion; scale-up; seed-train; microcarrier.

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O31. Design of an Electronic Nose for Odours Identification

[1*]Pádua, A. C. C. S., [2]Gruber, J., [3]Gamboa, H. & [1]Roque, A. C. A.

[1]UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia da Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[2]Departamento de Química Fundamental, Instituto de Química da Universidade de São Paulo,
Av. Prof. Lineu Prestes, 748 CEP 05508-000, São Paulo, SP, Brasil
[3]Laboratório de Instrumentação Engenharia Biomédica e Física da Radiação (LIBPhys-UNL), Departamento de Física, Faculdade de Ciências e Tecnologia da Universidade NOVA de Lisboa
**lead presenter: ac.padua@campus.fct.unl.pt*

Volatile Organic Compounds (VOCs) have been associated to certain diseases, food quality, and safety issues [1]. Sophisticated analytical techniques are the standard used for their characterization. However, they require specialized operation, time-consuming sample-preparation, and high operating costs. Other VOCs sensing devices, named electronic noses (E-noses), have been developed in the past decades [2]. They are easier to operate, faster, and low-cost.
This work is about the design and evolution of an E-nose based on opto-electronic sensors, which possess innovative sensing materials developed by our research group [3]. Three optical-device versions, one electrical-device, and a common system architecture [4] were assembled. Each E-nose version was characterized, its limitations were identified, and then optimized for the next version. The analysis time, signal-to-noise ratio, and stability were some of the parameters improved [5].
A proof of concept was performed with E-nose V1. E-nose V2 was mainly used for reproducibility tests, and to study the impact of the sensing film's production technique on the capacity for VOCs classification. Diverse machine learning algorithms were explored in this process for features extraction and selection, and for classification. Moreover, the sensing films' stability was assessed by morphological evaluation before and after exposure to specific VOCs.
The proof-of-concept showed that it is possible to distinguish the 13 pure VOCs tested with 100 % accuracy. The reproducibility tests performed with E-nose V2 indicated the sensing film's lifetime. Moreover, both methods tested for sensing film's production are adequate, since the estimated true error of classification was inferior to 6 % for all the classification tools. Overall, we achieved a miniaturized, accurate and easy-to-use E-nose system, well-characterized, and that can be explored towards diverse applications, such as medical research, food industry, and security.

Keywords: Electronic nose; Volatile Organic Compounds; Machine Learning.

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O32. Insulin-degrading enzyme inhibition by nitric oxide overproduction directly impairs glucose tolerance driven by hyperinsulinemia

[1*]Borges, D.O., [2]Oliveira, R.M.O., [3]Penha-Gonçalves, C. & [2]Macedo, M.P.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*
[2]*Chronic Diseases Research Center – CEDOC/NOVA Medical School – Universidade NOVA de Lisboa, Rua Câmara Pestana, n.6, 6-A, Lisbon, Portugal*
[3]*Instituto Gulbenkian de Ciência, Rua da Quinta Grande, n.6, Oeiras, Portugal*
**lead presenter: borges.biomed@gmail.com*

Insulin degrading enzyme (IDE) gene variants were recently added to the susceptibility map of metabolic diseases[1]. However, pathophysiology changes related to life style interventions were poorly explored. Diet induced obesity (DIO) is characterized by a subclinical inflammatory state, where nitric oxide (NO) is one of the key players[2]. Although NO was already described as modulator of IDE activity[3], evidences about the insulin degradation impairment in livers or hepatocytes under subclinical inflammation are unveil. Hypothesis: We hypothesised that DIO or *in vitro* increase in liver NO content played by inducible NO synthase (iNOS) will inhibit IDE activity. Moreover, in IDE knockout (KO) models we expect to see a similar hyperinsulinemia and glucose intolerant phenotype as in wild types under DIO. Methods: Male C57Bl/6J control, IDE KO and Liver-IDE-KO mice underwent through normal diet or DIO (12 weeks). Oral glucose tolerance test was performed. Liver iNOS and IDE expression was assessed as well as IDE activity and liver NO content. *In vitro*, a hepatocyte cell line (HEPA 1-6) was transfected with a plasmid to overexpress iNOS. Cells were stimulated with insulin and iNOS and IDE expression measured by western blot. IDE activity was also evaluated. Results: Global and Liver specific ablation of IDE drove glucose intolerance. Hyperinsulinemia was also observed in both models, as well as in DIO groups, where liver IDE activity was impaired. The overexpression of iNOS in hepatocytes also showed a decrease in IDE activity. Conclusions: Our results indicate that genetic IDE absence or IDE impairment due to lifestyle interventions are common features of glucose intolerant state. From mouse to cells, we also conclude that increased iNOS expression is direct related to IDE activity inhibition. Altogether, we conclude this is an important mechanism in the transition of normal to hyperinsulinemic states, which can further lead to insulin resistance in metabolic diseases.

Keywords: Insulin Resistance; High fat diet; iNOS.

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O33. Gold Nanoparticles Irradiated with a Green Laser as a Tool for Cancer Treatment

[1]*Pedrosa, P., [1]Baptista, P.V. & [1]Fernandes, A.

[1] *UCIBIO, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal;*
*lead presenter: *pm.pedrosa@campus.fct.unl.pt*

Nanoparticles are known for their capacity to passively accumulate in tumor site through enhanced permeability retention effect, being generally explored for chemotherapy since they locally increase the concentration of the chemotherapeutic [1,2]. Gold nanoparticles (AuNPs) have also photothermal capacities, converting electromagnetic radiation into heat. Photothermal therapy has been used to selectively destroy cancer cells while sparing healthy cells, which are more resistant to an increase in temperature. AuNPs are commonly coupled to near infrared laser for hyperthermia, promising high tissue penetration depth. In contrast, visible lasers affect only irradiated spots, but have increased photothermal efficiency, allow a visually guided irradiation, and have an increased effect on blood vessels, being used as tool in medical surgery for safe tissue ablation without bleeding and minimal destruction of surrounding tissue [3]. In our work, we show that the combination of 14nm diameter AuNPs with a 532nm CW laser can be an effective tool for hyperthermia of cancer cells [4]. An increased effect is observed when hyperthermia is combined with chemotherapy selectively killing both chemo sensitive and resistant cancer cells. We have also shown that the laser power can be tuned to only destroy blood vessels in the presence of AuNPs, and that irradiating AuNPs functionalized with an angiogenic peptide can not only precisely cauterize blood vessels but also humper the formation of new blood vessels in a chicken embryo model [5].
This opens the door for using visible lasers in cancer surgery and treatment, in combination with nanoparticle chemotherapy, possibly reducing side effects while preventing acquired resistances and neo-angiogenesis.

Keywords: Gold Nanoparticles; Green Laser; Cancer; Chemotherapy.

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POSTER PRESENTATIONS

P1. Unveiling the transcriptional regulation behind cell-specific gene expression of the *Setaria viridis PEPC1* promoter in Rice mesophyll cells

*[1]Carvalho, P., [2]Langdale, J. A. & [1]Saibo, N. J. M.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal*
[2]*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK*
*__*lead presenter__: pedro.carvalho@itqb.unl.pt*

Plant production is known to be highly associated with photosynthesis efficiency. At the present atmospheric CO_2, C3 plants, such as rice, have a photosynthesis efficiency much lower than C4 plants (e.g. maize, setaria). One way to increase rice yield is to implement the C4 metabolism in this C3 plant. C4 photosynthesis requires that key enzymes are expressed in a cell-specific manner. It has been reported that C4 promoters (e.g. maize *PEPC1* promoter) can drive cell-specific gene expression in rice [1], however, nothing is known about the transcription factors (TF) involved. In this work, we have studied the transcriptional regulation of the *Phosphoenol pyruvate carboxylase 1* gene promoter from *Setaria viridis* (proSvPEPC1) when transformed in rice. We have initially shown that both *SvPEPC1* promoter fragments, 2200 bp and 1100 bp upstream ATG, can drive mesophyll-specific gene expression in rice. To investigate the transcriptional regulation underlying this cell-specificity, we have used a yeast one-hybrid (Y1H) system to identify rice TFs binding to the 1100 bp fragment. The Y1H screening of rice cDNA expression libraries revealed only one TF interacting with this promoter. In order to identify more rice TFs binding to the pro*SvPEPC1*, we have used in silico tools, PlantPan2.0 [2] and PTFDB [3], to predict rice TFs that bind to the pro*SvPEPC1* and also to pro*ZmPEPC1*. By comparing both predictions, we identified four TFs that bind to the *PEPC1* promoters of both maize and setaria and three that are different between these C4 species. We are now validating these results in vivo (Y1H). In conclusion, we have shown that 1100 bp of the pro*SvPEPC1* is enough to drive mesophyll-specific gene expression in rice. In addition, we identified eight rice TFs that bind to this region and may regulate the cell-specific gene expression seen in rice mesophyll cells.

Keywords: Mesophyll-specific gene expression; Transcriptional regulation; C3 to C4 photosynthesis.

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P2. The role of RIC proteins in *Staphylococcus aureus* and *Escherichia coli*

[1]*Silva, L.O. & [1]Saraiva, L.M.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*
**lead presenter: lsilva@itqb.unl.pt*

Host innate immune system produce reactive oxygen/nitrogen species that damage important cellular components of the pathogens. For instance, iron-sulfur (Fe-S) containing proteins, that take part in key metabolic pathways, are particularly susceptible to damage by these reactive species.
RIC (Repair of Iron Centres) proteins are a widespread family of bacterial di-iron proteins which are also present in pathogenic eukaryotes, such as *Trichomonas vaginalis*. We previously showed that in *Escherichia coli*, *Staphylococcus aureus* and *Neisseria gonorrhoeae* RIC protects aconitase and fumarase (two Fe-S enzymes of the TCA cycle) from oxidative and nitrosative stresses [1].
We have now examined the role of RIC in *S. aureus* resistance and virulence to the host immune cells [2]. We show that RIC protects *S. aureus* from the oxidative stress produced by murine macrophages and promotes internalization and survival in human epithelial lung cells. The function of RIC was also studied *in vivo* in the wax moth larvae *Galleria mellonella*, and the results revealed that a *S. aureus* strain deleted in *ric* has reduced survival during the infection of the host.
In *E. coli*, a Bacterial Two Hybrid screening was used to search for RIC-protein interaction partners [3]. The DNA-binding protein from starved cells (Dps) was identified, and the potential interaction was further proved by Bimolecular Fluorescence Complementation and pulldown assays. *E. coli* Dps is an iron-storage protein with DNA-binding ability that belongs to the ferritin superfamily that also protects cells from oxidative stress. We further observed that the protein-protein interaction between RIC and Dps serves to protect *E. coli* from reactive oxygen species that are formed intracellularly.

Keywords: di-iron protein; RIC; oxidative stress; *Staphylococcus aureus*; *Escherichia coli*.

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P3. Pathogenic foodborne viruses in fish tissues: development of Multiplex *TaqMan* qPCR assays

[1]*Silva, A.F., [1]Nunes, M. & [1]Barreto Crespo, M.T.

[1]*iBET – Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; ITQB – Instituto de Tecnologia Experimental e Biológica, Oeiras, Portugal*
**lead presenter: andreiasilva@ibet.pt*

Food and food environments are a major source of viral transmission to humans, being norovirus (NoV) and Hepatitis A (HAV) the most frequently reported agents in foodborne outbreaks. In Portugal, virological control in food is still poorly made with no legislation making that control mandatory, namely in one highly prized food matrix in our country: fish. Portugal leads fish consumption rate among the EU, with an annual average consumption of 60 kg/capita, being fishing a cultural and traditional habit. Fishery has a high impact in the country's economy, being important to ensure its safety as food concerning virologic hazards.
Therefore the main goal of this study is to develop and optimized *Taqman* multiplex qPCR protocols for the identification/quantification of the referred viruses in four fish species, namely: gilthead seabream and sea bass, two of the most extensively farmed species of fish in the Portuguese aquacultures; the European pilchard, the main species used in the canning industry; and the horse mackerel, one of the most consumed fish species in the country. Also, 4 more viruses responsible for major economic losses in fisheries/aquaculture due the high morbidity/mortality will be analysed, namely: Infectious Pancreatic Necrosis Virus, Viral Haemorrhagic Septicaemia Virus, Infectious Hematopoietic Necrosis Virus, and Viral Nervous Necrosis Virus.
For this purpose, animal´s tissues samples were already dissected and RNA extraction was optimized using an in house protocol based in trizol extraction with alterations, for further application in qPCR protocols. These protocols are now optimized as singleplex for NoV GI, NoV GII, and HAV, and as a duplex for NoV GI and GII, being *in vitro* transcribed RNA transcripts used as template to generate standard curves. The limit of detection (LoD) ranged from 10^2 to 10 genome copies/µL. Regarding the 4 fish viruses the qPCR protocols optimization are still ongoing with the preparation of the standard curves.

Keywords: Foodborne viruses; Fish hazards; Multiplex *TaqMan* qPCR protocols.

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P4. Atomistic picture of charged metabolite – protein interaction with repercussions on protein stabilization

[1]*Silva, M., [1]Viegas, A., [1,2]Figueiredo, A.M. & [1]Cabrita, E.J.

[1]UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte de Caparica, Portugal
[2]Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London, WC1E 6BT, UK
**lead presenter: msi.silva@campus.fct.unl.pt*

Different environmental factors can change the composition of the protein environment and have been associated with protein misfolding and, ultimately, with neurodegenerative diseases such as Alzheimer's or Parkinson's.[1] One very important aspect is that the intracellular environment is extremely crowded with small charged metabolites that can form ion-pairs and have the potential to act on the folding and stability of proteins. Inspired by the high concentrations of organic charged metabolites found in the cell milieu, specifically the choline cation and the glutamate anion, and in our previous studies with imidazolium-based ionic liquids (IL), that disclosed the effects of specific P-IL interactions on protein stability,[2,3] we studied the effect of the novel biocompatible IL [Ch][Glu] on the stability, structure and dynamics of the globular domain B1 of protein G (GB1)[4] to achieve a complete mechanistic and molecular understanding of all the forces in play. The interaction was investigated using a combination of fluorescence and NMR spectroscopy. We show that the [Ch][Glu] effect on GB1 is a combination of ion-protein, ion-solvent and ion-pair interaction through weakly binding in charged and solvent accessible residues that contributes to the increase of the stability of secondary structure elements, as data from chemical shift perturbation, temperature dependence, protein dynamics, hydrogen exchange and water-protein contacts implies. We expect to contribute to an understanding of how changes in the cellular homeostasis may control the protein folding landscape.

Keywords: charged metabolites; choline-glutamate ionic liquid; protein GB1; NMR spectroscopy.

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P5. Functional characterization of drought-responsive RING E3 Ubiquitin ligases in rice

[1,3*]Melo, F.V., [1,2]Lourenço, T., [1]Oliveira, M.M. & [1]Saibo, N.J.M.

[1]*Instituto de Tecnologia Química e Biológica António Xavier/UNL; Av. da República, Oeiras, Lisboa, Portugal*
[2]*Instituto de Biologia Experimental e Tecnológica; Av. da República, Oeiras, Lisboa, Portugal*
[3]*Programa de Pós-Graduação Ciência para o Desenvolvimento, Instituto Gulbenkian de Ciência; Rua da Quinta Grande, 6, Oeiras, Lisboa, Portugal*
*lead presenter: *fmelo@itqb.unl.pt*

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population and its production is highly affected by various abiotic stresses, including high salinity, drought, extreme temperatures, and nutrient deprivation and toxicity. To cope with adverse environmental conditions, plants have evolved a range of molecular and physiological mechanisms. Among these, the post-translational ubiquitination/proteasome system (UPS) has emerged as an important mechanism underpinning stress response. In the UPS, the E3 ubiquitin ligase enzymes target specific proteins for degradation, thus modulating the plant proteome. The UPS has therefore emerged as a potential candidate for crop improvement programs. Among the multiple E3 ubiquitin ligase enzymes, and based on *in silico* studies and literature, we have identified 16 Really Interesting New Gene (RING) E3 ubiquitin ligases that appear to play a role in the rice response to several abiotic stresses. We have tested the expression pattern of these 16 genes in rice subjected to salt stress, drought, phosphate deficiency, and ABA treatment. Based on the results from this experiment, we selected two genes that showed to be highly induced by drought and ABA, and show a tissue-specific and diurnal regulation expression. To functionally characterize the selected genes, we are using the Gateway cloning technology to generate knockout (CRISPR/Cas 9) and overexpression tagged rice lines. To assess the mode of action of the selected E3 ubiquitin ligases, we will also use the yeast-two-hybrid system to screen two cDNA expression libraries (drought- and salt-induced) and thus identify their protein interactors. A better understanding of the molecular mechanisms underlying rice response to abiotic stresses will provide us with important insights for the development of abiotic stress-resistant crops.

Keywords: Abiotic stress; Ubiquitination; Proteasome; Oryza sativa.

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P6. First insights into cork oak-ambrosia beetle's invasive symbiosis interaction

[1,2]*Nones, S., [1,3]Cruz, L., [1]Bonifácio, L., [1]Trindade, C.S., [1]Simões, F., [1]Pimpão, M., [1]Preza, F., [1]Valdiviesso, T., [1,4]Matos, J., [5]Branco, M. & [1]Sousa, E.

[1]*Forest Research Unit, National Institute of Agricultural and Veterinary Research (INIAV), Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa , Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal*
[3]*Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal*
[4]*Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculty of Sciences, University of Lisbon, Lisbon, Portugal*
[5]*Centro de Estudos Florestais, Instituto Superior Agronomia, Universidade Técnica de Lisboa, Lisboa, PortugaL*
**leader presenter: stefano.nones@itqb.unl.pt*

Climate change induced temperature and drought conditions are affecting tree physiology along with insect and microbe ecology, developing unprecedented interactions. Invasive symbioses between ambrosia beetles and microbiota are rising as a new and currently uncontrollable threat to forest ecosystems worldwide. Cork oak forests (*Quercus suber* L.; Fagaceae) have a key ecological role in the Mediterranean Basin and are being affected by this process in the form of evident stands vigour loss that leads to a complex decline. In Portugal, the ambrosia beetle *Platypus cylindrus* (Platypodidae) has been linked to cork oak tree death since the 1980's. This insect coevolved to develop highly specialized body structures for symbionts transportation and employs a peculiar strategy of attack, involving adult insects in gallery excavation and microbes/fungi inoculation, whereas larvae feed upon ambrosia fungi. Our research aims at understanding in a comparative framework the whole mechanism of insect tree colonization (*i.e.* host plant attractiveness/defence), symbiont interactions (*e.g.* new complex relationships putatively responsible for the higher aggressivity of this insect in the last years), pest attack distributions and to develop effective eco-friendly control strategies. Briefly, the methodologies applied comprise the analysis of plant metabolites, plant transcriptomic tools, DNA sequencing of microbes, mycangia structure, GIS and biometric data. The present work highlights our first findings from insect histological observations, spatial distribution of pest attacks in an experimental field and microbiological diversity in the wood bored galleries, namely: (i) images showing female and male specialized mycangia from insects embedded in resin prior to cutting, staining and optical microscope observation, (ii) field data, and (iii) a first insight into selected bacteria analysed by 16S rRNA gene sequencing, chosen among the overly 40 isolated colony morphotypes.

Keywords: symbiosis; ambrosia beetle; cork oak.

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P7. A Novel RNA-Binding Protein Involved in ABA Signaling and Metabolism

[1,2]*Albuquerque-Martins, R., [2]Jones, A.M. & [1]Duque, P.

[1]*Instituto Gulbenkian de Ciência; Oeiras, Portugal*
[2]*Sainsbury Laboratory, Cambridge University; Cambridge, UK*
lead presenter: *rfmartins@igc.gulbenkian.pt*

Serine/arginine-rich (SR) proteins are major modulators of alternative splicing, a key posttranscriptional mechanism in eukaryotes that greatly expands the coding capacity of genomes and provides an important means of regulating gene expression. Although the functional relevance of alternative splicing in plant systems remains poorly understood, new evidence suggests that this process may be crucial in plant stress responses mediated by the abscisic acid (ABA) hormone. Indeed, loss-of-function mutants for several *Arabidopsis thaliana* SR proteins exhibit altered ABA sensitivity and metabolism. The overall goal of this project is to investigate mechanisms upstream and downstream of SR protein function and the *in planta* role of these RNA-binding proteins in ABA metabolism and dynamics. To identify the SR mutants exhibiting the most striking ABA defects during early plant development, a phenotypic analysis was performed using insertion mutants for 11 of the SR genes. Here we show that the *Arabidopsis rsz21-1* loss-of-function mutant displays ABA hyposensitivity at different stages of early seedling development, indicating that this protein acts as a positive regulator of ABA responses. Moreover, overinduction under salt stress of the NCED3 and CYP707A3 genes in the *rsz21* mutant suggests a role in ABA metabolism. The activation and function of this SR protein in ABA-specific responses will be assessed combining proteomics and transcriptomics approaches. FRET biosensor constructs coupled to live imaging will also be used to assess whether ABA levels and dynamics are affected by RSZ21 function. Elucidating the mechanisms governing posttranscriptional control of ABA biosynthesis and signaling and how the hormone activates this new RNA regulatory layer may open the way for the development of new strategies to improve plant tolerance to adverse environmental conditions.

Keywords; abscisic acid (ABA); alternative splicing; *Arabidopsis thaliana*; plant abiotic stress responses; SR proteins.

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P8. Oxygen-tolerant and Highly Active W-Formate Dehydrogenase for Photocatalytic Reduction of CO_2

[1*]Oliveira, A. R., [2]Miller, M., [2]Robinson, W. E., [3]Reisner, E. & [1]Pereira, I. A. C.

[1]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*

[2]*Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom*

**lead presenter: ana.rpo@itqb.unl.pt*

One of the most urgent and challenging issues facing our society is to reduce carbon dioxide levels. Developing a sustainable process to reduce CO_2 to formate can change its role from a pollutant to a valuable feedstock contributing to a carbon-neutral economy. Formate has high energy density, has low toxicity and it is a non-flammable liquid at room temperature. Therefore, it constitutes a safe way to store energy from renewable sources[1]. Formate dehydrogenases – Fdh – are the enzymes responsible for reduction of CO_2, and are promising green biocatalysts for production of formate. Contrary to other Fdh, the W-containing formate dehydrogenase (FdhAB) from the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough, is oxygen-tolerant: its purification can be performed aerobically, maintaining a high catalytic activity[2]. This makes it a strong candidate to be used in photocatalytic systems for CO_2 bio-reduction. Coupling this high efficient enzyme to a light-harvesting material allows the use of light to power the production of formate. We developed a homologous expression system for the *D. vulgaris* FdhAB, which allows us to engineer new variants towards the design of a more efficient enzyme and to unravel its mechanism. Here we report on the properties of the recombinant enzyme, and the ability of FdhAB to directly receive electrons from the dye-sensitised TiO_2 – highlighting its potential in a photocatalytic system for sustainable formate production[3].

Keywords: Formate dehydrogenase; CO_2 Reduction; Photocatalysis.

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P9. Small molecule from *Corema album* modulates αSynuclein aggregation in a yeast model of Parkinson's Disease

[1,2]*Rosado-Ramos, R., [3]Foito, A., [4]Sevillano, D.M., [5,6,7]Outeiro, T.F., [4]Ottens, M., [3]Stewart, D., [1,2]Menezes, R. & [1,2]Santos C.N.

[1]*Instituto de Biologia Experimental e Tecnológica, Portugal*
[2]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal*
[3]*Environmental and Biochemical Sciences, The James Hutton Institute, Scotland*
[4]*Department of Biotechnology, Delft University of Technology, Netherlands*
[5]*Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal*
[6]*CEDOC – Chronic Diseases Research Center, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal*
[7]*Department of NeuroDegeneration and Restorative Research, University Medical Center Göttingen, Göttingen, Germany*
lead presenter: rramos@ibet.pt

Parkinson's Disease (PD) is the most common neurodegenerative movement disorder affecting millions of people worldwide. The lack of effective therapeutic strategies for the disease led to an increased burden in the healthcare systems giving to PD a public health priority status. Phytochemicals, particularly (poly)phenols, have emerged as potent lead molecules targeting several pathological processes underlying neurodegeneration. Our previous work shown that a (poly)phenol-enriched fraction (PEF) of *Corema album* leaves modulates central events in PD pathogenesis, namely αSynuclein aggregation and clearance. Attempting to identify potential bioactive compounds, the *C. album* PEF was submitted to a bio-guided fractionation using a well-established yeast model of αSynuclein aggregation. This approach led to the identification of compound CAL_X, whose molecular mechanisms underlying its protection was further investigated. As a pure small molecule, CAL_X was shown to reverse αSynuclein cytotoxicity by reducing the number of cells displaying αSynuclein aggregates and the size of aggregates as revealed by flow cytometry, fluorescence microscopy and filter-trap assays, respectively. Noteworthy, these effects were not mediated by the decrease of αSynuclein protein levels. Most importantly, it will be of great interest to investigate the potential role of CAL_X on the modulation of proteasome activity considering that it was also shown to interfere with αSynuclein aggregation kinetics. These data open new venues for the exploitation of CAL_X as a lead molecule for PD therapeutics.

Keywords: alpha-synuclein; aggregation; small molecule; Parkinson's Disease.

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P10. Search for 3D representative models of tumor microenvironment: challenges around endothelial cells

[1]*Franchi-Mendes,T., [1]Brito,C.

[1]*iBET, Instituto de Biologia Experimental e Tecnológica, Lisbon, Portugal*
**lead presenter: mtmendes@ibet.pt*

Tumor microenvironment (TME) is involved in tumor progression, invasion and drug resistance, it comprises extracellular matrix and several non malignant cell types[1]. In vitro tumor models have relied on 2D approaches using only tumor cells, though the importance of including TME components is well recognized. Therefore, 3D models depicting TME elements are being developed, still most use animal-derived materials such as Matrigel, lack longer culture duration and inclusion of multiple cell types[2].
Previous work from our group established 3D tumor models with fibroblasts and immune cells using an alginate encapsulation and orbital shaking strategy[3,4].
In this work, we propose to build an even more recapitulative TME by incorporating endothelial cells (ECs). For this, different strategies were tested.
Encapsulation using tumor aggregates of breast cancer cell lines, BT474 and HCC1954, or lung cancer cell line, H157, with ECs and fibroblasts was not able to preserve ECs, showing EC death early in culture.
A different approach combining tumor, fibroblasts and ECs in a unique multicellular spheroid was successfully generated in low adherence 96-well plate and then transferred to agitation-based systems. Depending on the tumor cell line, ECs were maintained or not along culture time (28 days). Interestingly, ECs were only observed in the presence of fibroblasts with evidence of co-localization, suggesting the importance of fibroblast derived products to sustain EC survival and this being a necessary requirement but not sufficient as it is also dependent on the tumor cells.
Different media were also tested, obtaining best results at day 28 when using VEGF and FGF-2. Currently, hypoxic culture mimicking conditions are being tested to overcome the need of these factors.
These preliminary results explored features required for EC maintenance in long-term tumor co-cultures. Future work includes assessing EC angiogenic capacity, drug challenge and study therapeutic resistance.

Keywords: 3D; tumor microenvironment; endothelial cells; oncobiotechnology.

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P11. HdrABC heterodisulfide reductase as a putative electron donor in dissimilatory sulfite reduction

[1*]Ferreira, D., [1]Venceslau, S.S., [2]Dahl, C. & [1]Pereira, I.A.C.

[1]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVA de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
[2]*Institut für Mikrobiologie & Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany*
**lead presenter: dferreira@itqb.unl.pt*

Sulfate-reducing organisms are important anaerobes for the biogeochemistry of the Earth by performing the dissimilatory reduction of sulfate, an essential reaction for the global sulfur and carbon cycles. In this pathway, sulfate is phosphorylated and reduced to sulfite, which is then reduced by the dissimilatory sulfite reductase DsrAB together with its cosubstrate DsrC to form a trisulfide in DsrC, that is finally reduced to sulfide [1]. The heterodisulfide reductase HdrABC is a protein complex widespread among anaerobic organisms, and is suggested to also be able to reduce the DsrC trisulfide, although its role has not been fully clarified [2].
In the sulfate-reducer *Desulfovibrio vulgaris* Hildenborough, HdrABC is associated with the flavin oxidoreductase complex FlxABCD, and plays an essential role when using ethanol as electron source [2]. The FlxABCD complex is proposed to oxidize NADH that reduces the HdrA flavin subunit and, through a flavin-based electron bifurcation (FBEB) mechanism, HdrABC couples the reduction of ferredoxin (endergonic reaction) with the reduction of the DsrC-bound trisulfide (exergonic reaction) [1-3]. For this last reduction to occur, the electrons flow from HdrA to HdrB, a subunit containing two recently characterized non-cubane [4Fe–4S] centers suggested to catalyze the reduction of the DsrC trisulfide [2,3]. In the case of *Archaeoglobus fulgidus*, the [NiFe]-hydrogenase complex MvhAGD is present instead of FlxABCD as an interacting partner of HdrABC, linking hydrogen oxidation with the DsrC-trisulfide reduction [4].
In this study, we describe studies on the role of the FlxABCD-HdrABC complex and its heterologous expression and purification from *D. vulgaris* and *A. fulgidus*, and respective spectroscopic characterization, towards *in vitro* studies of the FBEB process and DsrC trisulfide reduction.

Keywords: sulfate reducing organisms; anaerobic respiration; energy metabolism; electron bifurcation.

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P12. SnRK1 regulates the shoot apical meristem homeostasis in response to light

[1,3*] Fernandes-Lopes, F., [1,2] Landrein, B., [1] Jönsson, H. & [3] Baena-Gonzalez, E.

[1] *Sainsbury Laboratory-University of Cambridge, CB2 1LR, Cambridge, United Kingdom*
[2] *Laboratoire Reproduction et Développement des Plantes - ENS de Lyon, 46, allée d'Italie, Lyon, France*
[3] *Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, Oeiras, Portugal*
**lead presenter: filipa.lopes@slcu.cam.ac.uk*

In higher plants, the stem cells localized in the shoot apical meristem (SAM) are the source of all aboveground tissues. Over the past few decades, significant progress has been made to elucidate SAM organisation and function, and the key mechanisms that regulate the stem cell homeostasis. However, further studies are still needed to understand how environmental and metabolic signals modulate SAM activity in order to adjust plant growth to a particular environment.
The SnRK1 kinase is pivotal for readjusting cellular metabolism in situations of energy deficit, shifting the cell's metabolism from anabolic towards a catabolic mode, and ultimately down-regulating growth. Indeed, SnRK1 acts as a metabolic sensor maintaining energy homeostasis for plant growth and survival, and thus, is a potential candidate for modulating meristem activity in response to the environment and nutritional signals.
By using a combination of reverse genetics and advanced imaging technique, this project aims at the characterization of SnRK1 impact in the SAM and to gain insight into the underlying molecular mechanisms. Our preliminary data show already that SnRK1 is enriched in meristematic tissues and that plants lacking or overexpressing SnRK1 have altered meristem shape in response to different light intensities. Additionally, here we suggest that this response to light is due to SnRK1 impact in main meristem regulators, such as STM transcription factor and/or cytokinin pathway.

Keywords: SnRK1; Shoot apical meristem; Light

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P13. Susceptibility, resistance and all in between – assessing *Lathyrus sativus* responses when challenged with powdery mildew and rust fungi

[1]*Martins, D.C., [1]Santos C., [1]Araújo S.S., [2]Rubiales D. & [1]Vaz Patto M.C.

[1]ITQB NOVA; Av. da República, Oeiras, Portugal; dcmartins@itqb.unl.pt, css@itqb.unl.pt, saraujo@itqb.unl.pt, cpatto@itqb.unl.pt
[2]Instituto de Agricultura Sostenible, CSIC; Av. Menedez Pidal, Cordoba, Spain; diego.rubiales@ias.csic.es
**lead presenter: dcmartins@itqb.unl.pt*

Lathyrus sativus (grass pea) is a promising source of proteins and calories for drought-prone and marginal areas. Little is known on the availability of resistance in grass pea against powdery mildew and rust, two important foliar diseases affecting legumes worldwide [1]. *Erysiphe pisi* and *Uromyces pisi*, causal agents of pea powdery mildew and rust respectively, have been described to cause damage in grass pea [2].. More recently, *E. trifolii* was confirmed to overcome *E. pisi* resistance genes in pea [3]. Given the phylogenetic proximity, a comparable situation might be occurring in grass pea. In the present work we compared the range of grass pea responses against different powdery mildew and rust isolates, identifying new sources of resistance.
A worldwide grass pea collection (180 accessions), was repetitively inoculated under controlled conditions with *U. pisi*, *E. pisi* and *E. trifolii*, assessing disease severity (DS) and infection type (IT) [4]. High levels of resistance (DS≤10%) against *E. pisi* were identified on the majority of the accessions, with only 6% of them showing some degree of susceptibility. Conversely, half of the accessions were susceptible (DS≥30%) to *E. trifolii*, with only a quarter of them depicting high resistance. *E. pisi* and *E. trifolii* DS scores were weakly correlated (r=0,36), anticipating differences in the genetic control of the resistance against each pathogen. Grass pea rust DS levels showed a continuous distribution, with a compatible reaction (IT=4, and high DS, 30-40%) in the majority of the accessions. More interestingly, sources of partial resistance (low DS and high IT) were observed against all three pathogens. Partial resistance is considered to result in durable resistance, thus a valuable resource for crop protection [5].
Future research will focus on the study of the identified resistance mechanisms and respective underlying genes for their efficient use in breeding programs in grass pea and other phylogenetically close legumes.

Keywords: *Erysiphe pisi*; *Erysiphe trifolii*; *Uromyces pisi*; grass pea; disease resistance.

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P14. *Lathyrus sativus* agronomic performance: how does it depend on genotype and/or environmental conditions?

[1]Gonçalves L., [1]Pereira F., [1]Alves M., [2]Lourenço M., [3]Julião N., [4]Rubiales D., [1]Vaz Patto M.C.

[1]*ITQB – Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, Oeiras, Portugal*
[2]*Câmara Municipal de Alvaiázere, Praça do Município, Alvaiázere, Portugal*
[3]*Simões & Ramos L[da], Pussos, Alvaiázere, Portugal*
[4]*Institute for Sustainable Agriculture, CSIC, Cordoba, Spain*
**lead presenter: leticeg@itqb.unl.pt*

Grass pea *(Lathyrus sativus*) is an underused crop with a great breeding potential. It presents a high genetic diversity and can be source of novel genes for plant improvement programs. In Portugal, is part of the traditional heritage of dryland communities and is helping to revitalize dry agricultural regions such as Alvaiázere.
To understand the genotype and environmental influence on grass pea agronomic performance, identifying interesting sources of important traits for this national region, we conducted for a period of two years a field comparison of a collection of 150 different grass pea accessions from all over the world (including representatives of the national grass pea germplasm). Field trials were established using an α-latice design participatory experiment involving farmers, researchers and the local government. Characterization was performed using thirteen quantitative traits and eight qualitative traits (related to inflorescence, stem, leaf, pod, seed, plant growth rate and biotic stress susceptibility).
Data from both growing seasons were submitted to a phenotypic analysis to estimate genetic variances between traits and heritability values; and to a principal component analysis (PCA). The variance component associated to differences between genotypes was bigger than the variance component attributed to the effect of Genotype × Environment interaction term, except for seeds with *Bruchus* and the number of pods produced per plant traits. Broad sense heritability value was highest for main branch height (89%) followed by weight of 100 seeds (88%).
The comparative multivariate assessment of the accessions behavior in this region, depicted a high diversity among the *L. sativus* accessions and a clear clustering of the Iberian accessions. It was also possible to discriminate outstanding for many of the traits that can be useful to select for putative parental lines to use in prospective breeding schemes.

Keywords: *Lathyrus sativus*; Genotype × Environment interaction; agronomic traits.

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P15. High-throughput screening strategy to solve the structure of an arabinofuranosyltransferase involved in cell wall synthesis in *Mycobacterium spp*

[1*†]Rodrigues, J., [2,3†]Tan, Y.Z., [1]Rosário, A.L., [3]Dandey, V.P., [3]Wei, H., [4]Kloss, B., [2]Clarke, O.B., [3,5]Potter, C.S., [3,5]Carragher, B., [2]Mancia, F. & [1]Archer, M.

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

[2]Department of Physiology and Cellular Biophysics, Columbia University, New York, USA.

[3]The National Resource for Automated Molecular Microscopy, Simons Electron Microscopy Center, New York Structural Biology Center, New York, USA.

[4]Center on Membrane Protein Production and Analysis, New York Structural Biology Center, New York, USA.

[5]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, USA.

†These authors contributed equally

Lead presenter: jose.rodrigues@itqb.unl.pt

Background: Tuberculosis (TB) remains one of the world's deadliest communicable diseases, according to the World Health Organization. The organism responsible for TB, *Mycobacterium tuberculosis*, has a unique cell wall structure of composed mainly of mycolic acids, arabinogalactan, peptidoglycan and lipoarabinomannan, that accounts for its unusual low permeability and resistance towards common antibiotics. Arabinosyltransferases (EmbA/B/C) and arabinofuranosyltransferases (AftA/B/C/D) are membrane proteins responsible for the synthesis of *M. tuberculosis* cell wall arabinogalactan and lipoarabinomannan. While the first-line anti-TB drug ethambutol (EMB) inhibit EmbA/B/C arabinosyltransferases, AftA/B/C/D arabinofuranosyltransferases are not inhibited by EMB, representing novel targets for the development of novel TB therapeutics. We aim to determine the 3D-structure of mycobacteria Arabinosyltransferases (Embs) and Arabinofuranosyltransferases (Afts) using a combined approach of X-ray Crystallography and Cryo- Electron Microscopy, to understand drug-resistance mechanisms and provide templates for the structure-guided design of a new generation of therapeutics against TB.

Results: We selected 96 AftA-D and EmbA-D genes among 14 *Mycobacteria* species, successfully cloned 56 candidate genes into pNYCOMPS N23 plasmid and their expression was tested in *Escherichia coli* BL21(DE3) pLysS-T1^R, from which 28 proteins were expressed. After scale-up production and optimization, 8 leading targets were selected for structural studies, and two preliminary 3D structures were solved by single particle Cryo-Electron Microscopy (cryo-EM) with data collected at New York Structural Biology Center (NYSBC). We present the structure of a mycobacterial AftD solved to 2.9 Å resolution.

Conclusion: The Cryo-EM structure of a mycobacterial glycosyltransferase provides insights into the catalytic activity of these proteins and may serve as a tool for future structure-based drug design efforts.

Keywords: Tuberculosis; Mycobacteria; Cell wall synthesis; Arabinofuranosyltransferases; Cryo-EM.

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P16. The combination of CG-MD and Fluorescence quenching measurements: a framework to study protein-quinone interaction

[1]*Sousa, F.M., [1]Sena, F.V., [1]Arnarez, C., [1]Melo, M.N. & [1]Pereira, M.

[1]*Instituto de Tecnologia Química e Biológica – António Xavier, Universidade Nova de Lisboa, Av. da República EAN, 2780-157 Oeiras, Portugal*
lead presenter: filipe.sousa@itqb.unl.pt

The need for energy is common to all biochemical reactions that regulate and maintain the electrochemical disequilibria which we call life. Organisms are able to harvest and store chemical energy, by generating a transmembrane difference in the electrochemical potential through their membrane bound respiratory chains. Monotopic quinone reductases are respiratory proteins which contribute indirectly to the generation of membrane potential by reducing the quinone pool without translocating charges across the membrane. Type II NADH:quinone oxidoreductases (NDH-2s) are a family of monotopic quinone reductases present in organisms belonging to the three domains of life.
This work will try to address protein-quinone interaction in different families of monotopic quinone reductases. It will do so by establishing a large spectrum Corse-Grain Molecular Dynamics (CG-MD) framework which allows exploring the structural features responsible for the protein-quinone interaction. Additionally, Fluorescence quenching studies focusing on the interaction of NDH-2 from *Staphylococcus aureus* (an increasingly relevant human pathogen) and its substrates are used as an experimental validation tool to the data generated by the CG-MD approach.
Preliminary results have shown a stable interaction of NDH-2 with the modulated *Staphylococcus aureus* lipid bilayer, as well as successful substrate binding events for different types of quinones, proving the applicability of this approach.

Keywords: Staphylococcus aureus; NDH-2; Corse Grain – Molecular Dynamics; Quinone; Cell Membrane.

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P17. Copper and fluconazole: exploiting synergies to create effective drugs

[1]*Gaspar-Cordeiro, A., [1]Leitão, M., [1]Petronilho, A., [1]Rodrigues-Pousada, C. & [1]Pimentel, C.

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*
lead presenter: carolinacordeiro@itqb.unl.pt

For centuries and until the advent of commercially available antimicrobials, copper has been widely used to prevent and treat microbial infections. Preliminary data from our laboratory suggests a synergistic effect between copper and the antifungal fluconazole against the non-pathogenic yeast *Saccharomyces cerevisiae*. Further reinforcing this synergistic association, we found that a mutant strain, *Sc Δfet3,* previously shown to accumulate high amounts of copper [1], is hypersensitive to fluconazole. Because invasive fungal infections caused by opportunistic *Candida sp*. represent the most common fungal diseases among hospitalized patients receiving immunosuppressive or intensive antibacterial therapies, we next tested whether such a potent association was observed against *Candida glabrata*. This yeast, while phylogenetically close to *S. cerevisiae* causes life-threatening infectious diseases with an associated mortality rate of up to 50%. Consistent with a synergistic effect of copper and fluconazole against *Candida glabrata*, by performing checkerboard assays, we observed that copper decreases the MIC_{50} for fluconazole. Differently from *S. cerevisiae*, however, *C. glabrata Δfet3* mutant is not sensitive to copper or hypersensitive to both drugs, indicating that a different mechanisms of copper homeostasis is operational in this organism.
Based on the above findings we explored whether the coordination of Cu to fluconazole might result in a molecule with a higher fungicidal potential. The initial steps of complex synthesis and characterization pointed towards the formation of a complex with two fluconazole molecules coordinating one Cu(II) atom, which is currently being investigated.

Keywords: Copper; *Candida glabrata*; fluconazole; *Saccharomyces cerevisiae;* fungal infections.

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P18. Characterization of molecular features underlying drought tolerance in Mozambique's drought tolerant maize (*Zea mays* L.) varieties

[1,3]Miquitaio, D., [1]Luis, I.M., .[1]Alexandre, B.M, [2]Miguel, M.A., [1]Oliveira, M.M. & [1]Abreu, I.A.

[1]*Instituto de Tecnologia Química e Biológica (ITQB NOVA), Avenida da República 2780-157, Oeiras, Portugal.*
[2]*Instituto de Investigação Agrária de Moçambique, Centro Zonal Centro, C.P.42, Rua Pigivite 678, Chimoio, Moçambique.*
[3]*Universidade Pedagógica, Delegação de Manica, C.P.323, Bairro Heróis Moçambicanos, Chimoio, Moçambique.*
*<u>*lead presenter:</u> dizimalta@itqb.unl.pt*

Maize (*Zea mays* L.) is the most important staple food in Mozambique, but its production is low, mainly due to drought conditions. To overcome this tendency, it is important to invest on breeding programs to produce maize varieties that are resistant to drought stress. The principal aim of this work is to contribute with knowledge and important data that can be added to Mozambique's maize breeding programs, providing data on plant survival ability to drought stress, agronomic performance, and nutritional quality of the maize grain of three drought tolerant maize varieties commonly used by Mozambique´s farmers: Matuba, ZM309 and ZM523. Maize is a C4 plant that evolved a biochemical mechanism of concentrating CO_2 to overcome the oxygenase activity of Rubisco which makes C4 plants photosynthetically more efficient than C3 plants. We Hypothesize that the levels of photosynthesis enzymes (PEPC, PPDK, NADP-ME, and RubisCO) and their Post Translational Modifications (PTMs), such us phosphorylation and acetylation correlate with the photosynthetic capacity of maize plants under drought stress. These PTMs represent new potential targets for the development of novel genotypes with enhanced stress tolerance. To examine their potential role as molecular markers for drought tolerance, we will make use of cutting-edge proteomics techniques. Trials were conducted in growth chamber and greenhouse conditions at ITQB-NOVA at 25%, 40% and 75% of field capacity (FC), and also in field conditions in Mozambique. Preliminary data indicates that, under drought stress, Matuba uptakes more water, has reduced stomatal conductance, and performs higher photosynthesis rates when compared to ZM523 and ZM309. In field conditions, intercropping with bean, which is very common locally, did not improve maize productivity, probably due to competition between the two species.

Keywords: drought; maize; photosynthesis enzymes; post translational modifications.

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P19. Understanding Cutin Impact in Plant Immunity: Characterization of the Polymer by Solid State NMR

[1§*]Moreira, C., [1§]Bento, A., [1]Matzapetakis, M., [2]Ferreira, M. J. & [1]Silva Pereira, C.

[1]*Instituto de Tecnologia Química e Biológica António Xavier – Universidade Nova de Lisboa; Avenida da República 2780-157, Oeiras, Portugal;*
[2]*Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal.*
[§]*equally contributing authors*
*<u>lead presenter</u>: *carlosmoreira@itqb.unl.pt*

The cuticle is a hydrophobic barrier that covers the aerial parts of land plants. It is composed by a polyester matrix of cutin to which hydrophobic lipids associate. This polyester-based physical barrier is essential to protect plants against several biotic and abiotic stress conditions [1]. The chemical characterization of cutin usually implies the unspecific hydrolysis of the polymer [2], which successfully discloses its monomeric composition regardless that the structural organization is largely lost. Our team has developed a methodology to extract suberin – plant polyester that shares many similarities with cutin - that uses an ionic liquid in the dual roles of catalyst and solvent. The ionic liquid cleaves preferentially the acyl glycerol ester bonds in suberin (releasing glycerol). The process allows to recover suberin macromolecules preserving most of its native structure, which are able to reassemble *ex situ* as a water-proof biomaterial [3].
The present study aims to modify the process which we have established to extract suberin from cork to allow the extraction of cutin from tomato peels, regardless that the glycerol content of cutin is negligible compared to suberin. The spectroscopy and calorimetry data demonstrated the feasibility of the new process to recover the cutin polymer. The spectroscopic analyses showed that the recovered polymer is of high purity and preserves largely the chemical features of cutin recovered through dewaxing and enzymatic removal of polysaccharides. The thermal analyses suggest the formation of crystalline regions after the self-assembly of the polymer, hence the detection of a clear melting temperature.
We are unravelling the polymer chemistry, aiming to evaluate the potential of its composing oligomers to act as elicitors of plant immunity.

Keywords: Plant Polyesters; Cutin; Spectroscopy; Calorimetry; Ionic Liquids.

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P20. Evaluation of graft (in)compatibility in Grapevine

[1]*Tedesco, S., [2]Pina, A., [3]Kragler, F. & [1,4]Fevereiro, P.

[1]*Instituto de Tecnologia Química e Biológica António Xavier; Av. da República, Oeiras, Portugal*
[2] *Unidad de Hortofruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Instituto Agroalimentario de Aragón—IA2(CITA-Universidad de Zaragoza), Avda. Montanana 930, Zaragoza, Spain*
[3]*Max Planck Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, Golm, Germany*
[4]*Dpt. Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande 016, Lisboa, Portugal*
**lead presenter: sara.tedesco@itqb.unl.pt*

Bench mechanical graft at the nursery is nowadays the widest propagation method in viticulture and is the unique control-strategy against the devastating effects of *Phylloxera*. Nevertheless, grafting also constitutes a risk of disease dissemination and graft incompatibilities restrict the range of its application. Despite its importance, little information exists on grafting in *Vitis* spp. and, even less, on the incompatibility phenomenon. This work aims to elucidate the phenotypic parameters related to graft (in)compatibility among four grape cultivars and one resistant rootstock.
Homograft and heterograft combinations between scions of *V. vinifera* cultivar *Syrah* (clones 470 and 383) and Touriga Nacional (clones 112 and 21) and the rootstocks 110R (*V. Belandieri* x *V. Rupetris*) were performed at the nursery. The different graft combinations were analysed at the *callusing* stage, 21 days after grafting (DAG) and at the harvesting period (152 DAG). To quantify grafting success, following growth parameters were measured: *callus* development, root system development, length of the main shoot, rooting and sprouting rates and graft success rates. To assess (in)compatibility, chlorophyll fluorescent parameters and chlorophyll contents were measured in leaves from the different graft partners, the graft unions were internally characterized and field determination of compatibility constant (FCC) calculated.
Results are currently being analysed and will be discussed in terms of the genetic relationships between both graft partners (homografts and heterografts). The relationships between the phenotyping parameters will be evaluated and the degree of compatibility examined.

Keywords: *Vitis spp*.; grafting compatibility; grafting phenotyping; homo-heterografting.

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P21. Targeting human Cyclophilin D using Fragment-based Drug Discovery

[1]*Silva, D.O., [2]Matias, P.M., [2]Ventura, R., [1]Bandeiras, T.M. & [3]Graedler, U.

[1]*iBET-Instituto de Biologia Experimental e Tecnológica*
[2]*ITQB- Instituto de Tecnologia Química e Biológica (Universidade Nova de Lisboa)*
[3]*Merck KGaA - Molecular Interactions and Biophysics Group*
**lead presenter: dsilva@ibet.pt*

Cyclophilins are peptidyl-prolyl isomerases, catalyzing the cis-trans interconversion of proline in other proteins during protein folding [1]. Originally identified as the biological receptors for immunosuppressants such as cyclosporin, cyclophilins are abundantly and ubiquitously present in a wide range of tissue types and organisms [2]. Cyclophilin D (CypD) is the mitochondrial isoform of the enzyme, and a key regulator of the mitochondrial permeability transition pore [3]. Mitochondrial dysfunction has been implicated in a cascade of cellular processes linked to multiple sclerosis, neurological and cardiovascular diseases [3][4], making CypD a therapeutic drug target. Three different inhibitor/hit series including urea derivatives were discovered at Merck KGaA (Germany) using fragment-based SPR-screening and medicinal chemistry optimization. However, from the 58 SPR-confirmed hits, only 6 CypD-fragment crystal structures were obtained. Moreover, these hit series did not show sufficient cellular potency together with optimal pharmacokinetic properties and the project was abandoned by Merck.
In this work, two different mutants of CypD (hsCypD (K175I) and a double mutant hsCypD (K167Q, K175I)) are used to revisit the previous Merck hits that failed to co-crystallize or led to crystal structures with unfavorable crystal packing. Until now, CypD wild-type failed all attempts at crystallization. Successful expression, purification and crystallization of both CypD variants was achieved and their 3D structures determined. More recently, crystallographic structures of both variants in complex with fragments were obtained and these new structural insights will feed a new structure-based drug design campaign, hopefully leading to the synthesis of better ligand/more potent inhibitors of CypD.

Keywords: Cyclophilin D; Mitochondria regulation; Cardiovascular disease; Fragment-based drug discovery; Protein crystallization.

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P22. Connections between the SnRK1 carbon sensor and carbon metabolism

[1,2,3]*Peixoto, B., [3]Höhne, M., [3]Alexandre Moraes, T., [3]Stitt, M., [3]Lunn, J. & [1,2]Baena-González, E.

[1]*Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. Da República, 2780-157 Oeiras*
[3]*Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany*
**lead presenter: bpeixoto@igc.gulbenkian.pt*

Photosynthetic fixation of atmospheric CO_2 provides plants with all the necessary carbohydrates for sustaining growth and development. Starch metabolism, in particular, is a highly dynamic process, with synthesis and degradation rates being continuously adjusted to the ever changing light conditions in a way that starch is almost, but not completely, exhausted at dawn. The Snf1-related protein kinase 1 (SnRK1) is important for adapting cellular metabolism to low carbon conditions, largely by reprogramming gene expression and metabolism from an anabolic towards a catabolic mode to generate energy from alternative sources. Using yeast-two-hybrid and *in vitro* kinase assays, this kinase was also shown to interact with and regulate key enzymes of sucrose and starch metabolism, as well as class II trehalose-6-phosphate synthase (TPS) proteins, with potential regulatory roles in carbon metabolism. We are currently exploring whether SnRK1 regulates carbon metabolism *in planta* by analysing the metabolic profile of various SnRK1 gain- and loss of function mutants under steady state conditions, as well as in response to sudden environmental challenges. This is further complemented by the site-directed mutagenesis analysis and identification of SnRK1-target residues in these class II TPS proteins, which will be employed later in the generation of new phosphomimetic and phosphomutant plant lines. All in all, this work should determine whether the SnRK1 carbon sensor impacts specific aspects of carbon metabolism *in planta*. Furthermore it will reveal whether TPS proteins are implicated in this regulation and if not, provide novel insight into the function of these proteins.

Keywords: SnRK1; Carbon Metabolism; Starch; Sucrose.

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P23. Developing new molecular tools and models to study astrogliogenesis

[1]*Letra-Vilela, R.; [1]Cardoso, B.; [1]Murtinheira, F.; [1]Herrera, F.

[1]*ITQB-NOVA; Av. da República 2780-157, Oeiras, Portugal*
*lead presenter: *ricardo.letra.vilela@gmail.com*

The Central Nervous System (CNS) comprises different types of cells, including neurons, astroglia, oligodendroglia and microglia. Current evidence indicates that glial cells have an important role in CNS physiology and pathology. Astrocytes are the most common type of glial cells, and they change their normal conformation and grow in size in response to CNS insults. If the blood-brain barrier is compromised, astrocytes proliferate and form a glial scar. This scar protects the healthy tissue, but it remains long after the insult is over, blocking a possible regeneration of the damaged area. One of the canonical pathways involved in this response is the JAK/STAT3 pathway.
To understand this pathway and characterize it, we are developing molecular tools that will allow us to study key protein-protein interactions which are rate-limiting steps for JAK/STAT3 signaling. These molecular tools are based on a property of some proteins called protein complementation, that allows to split them in two or more non-functional fragments that will reconstitute the protein function when they are brought back together. In particular, we are using a split-Venus system to study STAT3 dimerization and protein-protein interactions, and creating a new possible protein complementation system with an optogenetic protein called miniSOG. This split-miniSOG system could be used to visualize protein-protein interactions at an ultrastructural level or to kill specific subpopulations of cells, such as cancer stem cells or reactive astrocytes.
We are also developing a model that allows the visualization of astroglial conformational changes by tagging GFAP, the main component of astrocytic intermediate filaments, with fluorescent and super-resolution tags. Our systems could contribute greatly to our understanding of astroglial biology in health and disease, but also to other biological phenomena involving JAK/STAT3 signaling.

Keywords: Astrocytes; JAK/STAT3; GFAP; Optogenetic; BiFC.

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P24. Development of green process technologies for the valorisation of fish canning waste streams

[1,2,3]*Rodrigues, L., [1]Pereira, C.V., [1]Gaspar, F.B., [4]Carvalho, A.M., [3]Duarte, A.R.C., [3]Paiva, A. & [1]Matias, A.A.

[1]*Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
[3]*Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Departamento de Química, REQUIMTE/CQFB, 2829-516 Caparica, Portugal*
[4]*Instituto Nacional de Investigação Agrária e Veterinária, I.P., Unidade de Tecnologia e Inovação, Av. da República, 2780-157 Oeiras, Portugal*
**lead presenter: liliana.rodrigues@ibet.pt*

The global canned seafood market is expected to reach 27.8 billion US dollars by 2025, with an annual growth of 3.2%. This industry is one of the oldest in Portugal and is expected to follow the global growing trend. However, being a fish-processing industry, it generates huge amounts of waste streams, accounting for 1.54 million tonnes/year in the EU, which will progressively increase with the market growth in the years to come. These residues represent an opportunity to convert low status wastes in valuable resources that can be used in a broad range of economically interesting applications. In fact, several studies point marine by-products as a remarkable source of bioactive molecules, such as lipids and proteins/peptides, which can hold a much higher market value than the original raw materials.
Within this context, in this work we aim at designing sustainable approaches to convert sardine residues, resulting from the Portuguese canning industry, into added value products. Our strategy combines the use of supercritical CO_2 and betaine-based deep eutectic solvents, for the extraction of fatty acids and proteins/peptides, respectively. The extraction processes were optimized by studying the effect of different process parameters on the recovery of target compounds, namely fatty acids and proteins, which were then quantified by GC-FID and UV-VIS, respectively. To evaluate the potential application of sardine extracts as bioactive ingredients, their cytotoxicity and anti-inflammatory activity were assessed in an intestinal cell model (CACO-2) and antiproliferative activity was evaluated using a colorectal adenocarcinoma cell line (HT29). Additionally, fatty acid extracts were screened for their antimicrobial activity on bacterial strains relevant to food and cosmetic applications.
The process technologies established in this work will allow the utilization of currently undervalued canned sardine waste streams to give origin to new health, food and cosmetic products.

Keywords: Fish canning waste streams; supercritical carbon dioxide; deep eutectic solvents; bioactive lipids and proteins/peptides.

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P25. Screen for novel *Chlamydia trachomatis* virulence proteins interfering with host cell trafficking pathways

[1*]Bugalhão, J.N., [1]Luís, M.P., [1]Vieira, A. & [1]Mota, L.J.

[1]*UCIBIO, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia da Universidade NOVA de Lisboa, Caparica, Portugal*
**lead presenter: joanabugalhao@fct.unl.pt*

Chlamydia trachomatis is the leading cause of sexually transmitted bacterial diseases. In women, it can cause infertility and ectopic pregnancy. This obligate intracellular human pathogen thrives within a membrane-bound compartment, the inclusion. *Chlamydia* manipulates host cell trafficking by using bacterial effector proteins delivered into the host cell by a type III secretion system. These effectors include inclusion membrane (Inc) proteins with important roles in *Chlamydia*-host cell interactions. We aimed to identify Incs subverting host vesicular trafficking and/or showing tropism for eukaryotic organelles using the yeast *Saccharomyces cerevisiae* as a model. We generated 60 yeast strains producing either the predicted cytosolic fragments of *C. trachomatis* Inc proteins fused to green fluorescent protein (Inc-GFP) or Inc-GFP proteins fused to the yeast SNARE Pep12 (Inc-GFP-Pep12), which anchors Inc fragments to the cytosolic side of endosomes. By performing vacuolar protein sorting assays (vps) [1] and fluorescence microscopy analysis, we identified two Incs causing vps defects in yeast, $IncZ_{91\text{-}215}$-GFP and $IncY_{192\text{-}268}$-GFP-Pep12. Furthermore, we found one Inc ($IncX_{1\text{-}88}$-GFP) showing tropism for lipid droplets (LDs), major organelles for the storage of neutral lipids and which are known to be recruited to the *Chlamydia* inclusion during infection [2]. We hypothesize that IncX might have an important role in this process and selected this protein for a detailed characterization. Using mammalian cells ectopically expressing truncated versions of IncX, we identified a hydrophobic domain within $IncX_{1\text{-}88}$ responsible for targeting the protein to LDs. In addition, co-immunoprecipitation assays showed that ectopically expressed $IncX_{139\text{-}189}$ binds 14-3-3β (phosphoserine-threonine binding protein). We are currently generating a *C. trachomatis incX* null mutant strain aiming to understand the biological role of IncX during infection.

Keywords: host-pathogen interactions; *Chlamydia trachomatis*; Inc proteins; vesicular trafficking; lipid droplets.

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P26. Iron piracy in Gram-negative bacteria: Unravelling the mechanisms of ferric-siderophore reduction

[1]Trindade, I.B. *, [1]Rollo, F., [1]Didier, I., [2]Masaki F., [3]Matias. P.M, [1]Moe, E. & [1]Louro, R.O.

[1]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Universidade Nova de Lisboa, Av. da Republica (EAN), 2780-157 Oeiras, Portugal*
[2]*Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minatocho, Hakodate, Hokkaido 041-8611, Japan*
[3] *Instituto de Biologia Experimental e Tecnológica (iBET), Apartado 12, 2780-901 Oeiras, Portugal*
**lead presenter: ines.trindade@itqb.unl.pt*

Iron is an essential element to virtually all organisms. However, the bioavailability of iron is limited, making iron a nutrient worth battling for. Iron is insoluble in an oxygenated atmosphere, and inside the host, most of it is incorporated into proteins to avoid the deleterious production of free oxygen radicals [1]. Therefore, in order to survive, microorganisms developed efficient strategies for iron scavenging. Amongst them, the utilization of siderophores is of upmost significance [2].
Siderophores are small molecular weight compounds with high affinity for ferric iron. They are extracellularly released in the apo-form, and then incorporated back as ferric-siderophores via specific receptors. The siderophore pathway finishes with the release of iron from the complex followed by its incorporation in the cell metabolism. Iron release, however, at the molecular level is one of the least understood steps of the siderophore pathway. Two main iron release strategies have been identified: one involves the hydrolysis of the siderophore molecule while the other recycles it for further rounds of iron uptake; the later involves recycling the ferric-siderophore via the reduction of the ferric iron and this mechanism is mediated by siderophore-interacting proteins [3]. Two distinct families of these proteins have been identified: the ferric siderophore reductase (FSRs) family and the Siderophore-Interacting Protein (SIPs) family. FSRs contain an unusual 2Fe-2S cluster and can use ferredoxin as electron donors, whereas SIPs contain flavin cofactors (FAD or FMN) and use NADH and/or NADPH as electron donors [4, 5].
Here we explore ferric-siderophore reduction in Gram-negative bacteria by performing the structural and functional characterization of representative SIPs and FSRs from species of the genus of *Escherichia* and *Shewanella*. Furthermore, we also explore the role of iron speciation in the different siderophores produced by these microorganisms.

Keywords: iron uptake; Gram-negative bacteria; siderophores; ferric-siderophore reduction; electron transfer.

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P27. Dissecting mechanisms of innate immune evasion in a zebrafish xenograft model

[1*]Póvoa, V., [1]Gil, M.M., [1]Fuzeta, M. & [1]Fior, R.

[1]*Champalimaud Centre for the Unknown, Av. De Brasília, Doca de Pedrouços, 1400-038 Lisbon, Portugal*
**lead presenter: vanda.povoa@neuro.fchampalimaud.org*

During tumorigenesis, cancer-immune system interactions are crucial to determine the outcome of the disease. While the more immunogenic tumor clones may be eliminated, the less ones may circumvent the immune response and expand in an uncontrolled manner in the immunocompetent host. Our previous studies (1) revealed that a pair of colorectal cancer cell lines derived from the same patient, SW480 (primary tumor) and SW620 (lymph node metastasis), exhibited contrasting engraftment rates in the zebrafish-xenograft model (~35% vs 90%). However, when we mix both, SW480 were now able to engraft (~64%). We hypothesize that SW620 induces an immunosuppressive environment that allow immune evasion and protects SW480 from being cleared. To uncover the cellular and molecular mechanisms that govern SW480-SW620 communication and their interplay with innate immunity, we are currently characterizing the immune cell compartment and molecules involved in the tumor rejection/maintenance. Our data shows that SW480 recruits more efficiently neutrophils and macrophages than SW620. To test whether innate immunity is involved in graft rejection, we suppressed it genetically (mutants) and observed that SW480 engraftment indeed increases, implying a major role for innate cells in tumor rejection. By manipulating the proportion of the 2 cell lines, we found that SW620 cell number correlates positively with engraftment, tumor size and proliferation. Moreover, the presence of SW620 in the mix leads to a reduction of neutrophils/macrophages recruitment. We are currently investigating through RNAseq which signaling molecules are involved and that may unveil new targets and strategies for immunotherapy.

Keywords: cancer; zebrafish-xenograft; immune evasion; innate immunity; tumor rejection.

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P28. Development of an anti-HER2 phage as a tool for monitoring untargeted phage display selections on breast cancer models

[1,2*]Moita, M.R., [1]Simão, D., [1]Silva, G.M, [1]Soares, H., [3]Hoet, R. [1,2]Brito, C. & [1,4]Barbas, A.

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901, Oeiras, Portugal
[2]ITQB, Instituto de Tecnologia Química e Biológica António Xavier, Av. da República 2780-157, Oeiras, Portugal
[3]Department of Pharmacology and Personalised Medicine, Maastricht University, Universiteitssingel 40, 6229 ER, Maastricht, Netherlands
[4]Bayer Portugal, Rua da Quinta do Pinheiro 5, 2790-012, Carnaxide, Portugal
**lead presenter: mraquelmoita@ibet.pt*

Phage display is a robust technology for antibody discovery, providing also the opportunity to identify novel antibody targetable antigens[1]. This technology is based on highly diverse libraries of phages displaying antibody fragments on its surface, allowing the isolation of specific binders to virtually any target by an affinity selection process called panning. Many protocols have been proposed for targeted strategies. However, for untargeted approaches aiming at the identification of novel targets in cells or tissues, only few successful cases have been reported, highlighting the need to develop new panning methods for this purpose[2].
Our goal is to establish a protocol of cell-based untargeted phage display for the selection of breast cancer-relevant antibody candidates. For this we will take advantage of 3D breast cancer cell models, which have been shown to recapitulate important disease features not depicted in monolayer cultures[3], [4]. Here, we describe the development of a phage particle displaying the single chain variable fragment (scFv) of Herceptin, which targets the human epidermal growth factor receptor 2 (HER2). This phage will be used to spike the Tomlinson I+J library, working as an internal control to assess panning efficiency in $HER2^{+}$ cancer cells.
The phagemid in which the Tomlinson I+J library is expressed, pIT2, was fully-sequenced and the scFv sequence of Herceptin was cloned into it. To allow the discrimination between Herceptin-phage and the library phages, the ampicillin resistance gene on the phagemid was exchanged for a tetracycline resistance gene. Both phagemids, either with ampicillin or tetracycline resistance, were cloned into the *E. Coli* TG1 for phage production. Flow cytometry analysis on $HER2^{+}$ and $HER2^{-}$ breast cancer cell lines confirmed the specific binding of the Herceptin phage to $HER2^{+}$ cells. This tool is now being used for the optimization of a phage display selection protocol against $HER2^{+}$ breast cancer cells from 2D and 3D cultures.

Keywords: Antibody discovery; Breast cancer; Phage display; Phenotypic screening; 3D cell culture.

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P29. Intercropping, irrigation and fertilizer impact in soil quality and grain mineral content in field grown maize

[1*]Custódio, V., [2]Amorós, R., [3]Carranca, C., [4]Broadley, M.R. & [1]Oliveira, M.M.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Genomics of Plant Stress, Av. da República, 2780-157 Oeiras, Portugal
[2]Instituto Nacional de Investigação e Desenvolvimento Agrário (INIDA), CP 84, 7600 Praia, Cabo Verde
[3]Instituto Nacional de Investigação Agrária e Veterinária, Quinta do Marquês, Nova Oeiras, 2784-505 Oeiras, Portugal
[4]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
**lead presenter: valeria.custodio@itqb.unl.pt*

Maize (*Zea mays*) is the main source of macro- and micronutrients for most low-income rural and urban populations of Sahel region. However, maize grains typically contain inadequate amounts of essential dietary micronutrients such as iron (Fe), zinc (Zn) and selenium (Se). The concentration of these elements in the grain may however be modified using biofortification strategies. Fertilizers are one of the strategies used to enrich the plant in macro- (e.g. N, P, K, S, Mg) and micronutrients (e.g. Fe, Zn, Se) that play an important role in plant development, growth, nutrient allocation and productivity. Applications of fertilizers may lower the soil pH due to protons' release in nitrification process and may reduce the C/N ratio. These two soil features are important for microbial biomass and structure (bacteria and fungi). Our aim was to investigate how agricultural practices such as intercropping, irrigation, and fertilization may impact the soil quality and grain mineral concentration in maize grown in two Cabo Verde regions with different edaphoclimatic conditions. A field with 3 treatments (control, inorganic and organic fertilizers) in two different regions (arid and semi-arid) in Santiago Island (Cabo Verde) was set-up. Mineral concentrations were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS). The preliminary studies showed that the cropping system did not affect Fe, Zn or Se concentrations in maize grain produced in both edaphic regions. However, irrigation affected the Fe and Se concentrations in the grain, and mineral fertilization with ammonium sulfate caused a reduction in Se concentration. Present results also showed that green manure (*Leucaena* sp.) had no effect on Fe, Zn and Se concentrations in maize grain. Due to the importance of soil microbiome on plant development and mineral uptake efficiency, the natural microbiome in the two soils will be characterized, and will be related with some soil properties and maize crop development and grain quality, in order to provide recommendations regarding best maize agricultural practices for Cabo Verde.

Keywords: Fertilizers; biofortification; mineral nutrition; maize.

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P30. Analysis software for subcellular studies of protein localization and interactions by live cell microscopy

[1*]Saraiva, B.M., [1]Monteiro, J.M., [1]Pereira, A.R., [1]Reichman, N.T., [1]Tavares, A.C., [1]Figueiredo, J., [2]Krippahl, L., [3,4]Fernandes, F., [1]Filipe, S.R. & [1]Pinho, M.G.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
[2]CENTRIA, Dept. Informática, Faculdade Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[3]Centro de Química-Física Molecular and Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal
[4]iBB-Institute for Bioengineering and Biosciences, Biological Sciences Research Group, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal
*<u>*lead presenter*</u>: bsaraiva@itqb.unl.pt*

Over the last decades live-cell microscopy has revolutionized the way we look at organisms. Bacteria in particular are no longer seen as “bag of enzymes”, but rather an organized cell, where protein localization is spatially and temporally controlled. With development of better labelling methods and novel microscopy techniques users can now look at important subcellular structures and acquire large datasets of microscopy images. But while these techniques empower the user to gain knowledge on cellular processes they also create a new problem as the generated data is difficult to analyse in a reasonable time using non-automated tools.
In order to speed microscopy image analysis, we have developed eHooke, a user-friendly software, that is capable of automatic identification, segmentation and measurement of fluorescence signal in different shaped bacteria. Furthermore, we also show how these methods for cell identification and segmentation can be combined with the automatic calculation of Pearson’s Correlation Coefficients (PCC) and Förster Ressonance Energy Transfer (FRET) to gain new insights about protein interactions in live bacterial cells.

Keywords: Bacteria; Live Cell Imaging; Image Analysis; Protein Localization; Protein Interactions.

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P31. Signaling molecules triggered by acute injury in the *Drosophila melanogaster* adult brain

[1]*Rodriguez, A., [1]Alves, C. & [1]Rhiner, C.

[1]*Champalimaud Centre for the Unknown; Lisbon, Portugal*
**lead presenter: anabel.rodriguez@neuro.fchampalimaud.org*

In the mammalian adult brain, neurogenesis occurs throughout life in restricted regions and it contributes to neural plasticity, damage repair and regeneration. Damage due to brain injuries leads to the activation of quiescent adult neural stem cells and initiates regenerative neurogenesis. The ability to regenerate is observed in varying degrees in many organisms. For example, vertebrates like amphibians and fish have high regenerative capacity, but mammals have restricted ability to regenerate in adult stages. In general, the regulation of damage-induced stem cell activation is poorly understood. Only recently in Drosophila melanogaster damage-induced neurogenesis have been detected in the optic lobes. Which signaling molecules are involved in the activation of these stem cells close to injury? A whole genome profiling (microarray) performed in damaged versus undamaged brains showed upregulation of genes involved in stress, immune and defense responses. We are currently performing RNAi of genes implicated in these pathways in the adult brain to test if they are involved in stem cell activation. What happens when we block these major signaling responses? We are going to use chemical inhibitors applied in the fly food to shut down these responses and evaluate the effect on stem cell proliferation. Finally, we are also going to perform acute injury in bacteria-depleted flies under sterile conditions to evaluate the contribution of bacterial infection to kick-start tissue regeneration.

Keywords: damage-induced neurogenesis; stem cell activation; signaling molecules.

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P32. Fully-humanized Skin-on-a-chip for medical applications

[1,2]*Zoio, P.A, [1]Oliva, A.G., [2]Conde, J.P., [1]Diogo, V.S.

[1]*Instituto de Tecnologia Química e Biológica António Xavier; Av. da República 2775-412, Oeiras, Portugal;*
[2]*INESC Microsistemas e Nanotecnologias; R. Alves Redol 9, 1000-029, Lisboa, Portugal;*
lead presenter: *pattie.zoio@gmail.com*

In vitro human skin models are gaining attention for their importance as tools for basic research and for the pharmaceutical and cosmetic industries. However, current commercially available human skin models are only suitable for short-term studies due to the time-dependent contraction of their collagen gel-based matrix. Other limitations include the use of non-human extracellular matrix components and lack of dynamical flow systems and mechanical forces.
In recent years, advances in biomaterials and microfluidics technology made it possible for the culture of artificial skin to move a step ahead giving rise to the development of microfluidic skin-on-chip platforms. These systems are able to reproduce key aspects of the *in vivo* cellular microenvironment by including fluid flow and finely tuned forces.
This project aims at developing an innovative microfluidic system to grow and sustain a physiologically relevant human skin model and to access its potential applications. Our approach begins with the production of a fully-humanized skin model by combining the production of a fibroblast derived matrix and the use of an inert porous scaffolds for long-term, stable cultivation, without using animal components. This technique is then combined with the use of a biomimetic "organ-on-a-chip" system which includes dynamic perfusion for continuous supply of nutrients and metabolites. Also, we present a reversibly sealed chip with a module-based architecture that provides an easy to use workflow, an efficient and precise cell seeding and a removable culture insert that can be transferred between modules.
We anticipate that this innovative platform will reduce the dependence on animal models and provide a new *in vitro* tissue system compatible with long-term studies to study skin diseases and evaluate the safety and efficacy of novel drugs and technologies.

Keywords: Skin-on-a-chip; Microfluids; *In vitro* skin models; Fibroblast derived matrix; Modular structure

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P33. Identification of *Streptococcus pneumoniae* by a real-time PCR assay targeting SP2020

[1*]Handem, S., [1]Tavares, D., [1]Carvalho, R.J., [1]Paulo, A.C., [2,3]de Lencastre, H., [4]Hinds, J., & [1,5]Sá-Leão, R.

[1]*Laboratory of Molecular Microbiology of Human Pathogens, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB-NOVA), Oeiras, Portugal.*
[2]*Laboratory of Molecular Genetics, ITQB-NOVA, Oeiras, Portugal.*
[3]*Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, NY, USA.*
[4]*Institute for Infection and Immunity, St George's University of London, London, UK.*
[5]*Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*
**lead presenter: sarahandem@itqb.unl.pt*

Streptococcus pneumoniae (the pneumococcus) colonizes asymptomatically the human nasopharynx. It is also responsible for high rates of morbidity and mortality worldwide mainly due to otitis media, pneumonia, bacteremia and meningitis.
Correct identification of pneumococci is therefore very important and not always straightforward, as pneumococci share the niche with closely related species of streptococci that frequently harbor homologues of pneumococcal specific genes.
Real-time PCR targeting *lytA* (the major autolysin gene) and *piaB* (permease gene of the *pia* iron uptake ABC transporter) are currently used as the gold-standard culture-independent assays for pneumococcal identification. We evaluated the performance of a new real-time PCR assay – targeting SP2020 (encoding a putative transcriptional regulator gene) – and compared its performance with previously described assays. A collection of 150 pneumococci, 433 non-pneumococci and 240 polymicrobial samples (obtained from nasopharynx, oropharynx, and saliva; 80 from each site) was tested. SP2020 and *lytA* assays had the best performance (sensitivity of 100% for each compared to 95.3% for *piaB*). The specificity for *lytA* and *piaB* was 99.5% and for SP2020 was 99.8%. Misidentifications occurred for the three genes: *lytA*, *piaB* and SP2020 were found sporadically in non-pneumococcal strains; and *piaB* was absent in some pneumococci. Combining *lytA* and SP2020 assays resulted in no misidentifications. Most polymicrobial samples (88.8%) yielded concordant results for the three molecular targets. The remaining samples seemed to contain non-typeable pneumococci (0.8%), and non-pneumococci positive for *lytA* (1.7%) or SP2020 (8.7%). We propose that combined detection of both *lytA*-CDC and SP2020 is an improved alternative for the identification of pneumococcus either in pure cultures or in polymicrobial samples.

Keywords: *Streptococcus pneumoniae*; Identification; Real-time PCR; Polymicrobial.

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P34. Fluorinated Ionic Liquids for Encapsulation of a Therapeutic Protein

[1]*Alves, M., [2]Martins,I.C., [2]Santos, N.C., [3]Leandro, P., [4]Rebelo, L.P., [4]Araújo, J.M.M., [4]Pereiro, A.B. & [1]Archer, M.

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Avenida da República, 2780-157 Oeiras, Portugal
[2]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal
[3]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
[4]LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
**lead presenter: marcia.alves@itqb.unl.pt*

Fluorinated Ionic Liquids (FILs), a new class of environmentally benign and tailor-made solvents, have surfactant properties and spontaneously self-assemble at concentrations above their critical aggregation concentration (CAC) [1]. Since these FILs are biocompatible and non toxic, they are ideal candidates for protein drug delivery systems in order to overcome problems such as route of administration, dosage, protein instability and degradation.
Our previous studies showed that the addition of ionic liquids had no significant effect on the stability, structure and activity of lysozyme [2]. A distinct behaviour was observed in dynamic light scattering experiments for non-surfactant and surfactant ILs, with the latter encapsulating the protein at concentrations above the CAC [2].
Phenylketonuria (PKU), the most frequent disorder of the amino acid metabolism, is related with the deficient activity of the enzyme Phenylalanine Hydroxylase (PAH). PAH metabolizes L-Phenylalanine (L-Phe) to L-Tyrosine (L-Tyr) in the liver, and its malfunction leads to intolerance to the nutritional intake of L-Phe. PKU patients suffer from severe psycho-motor impairment due to the toxic accumulation of L-Phe in the central nervous system, and low levels of of L-Phe derived biosynthesized neurotransmitters also contribute to negative clinical outcomes. [3]
In this work, our aim is to analyze the effect of FILs on the stability, function and structure of human PAH, a potentially therapeutic protein. Different techniques were used for this purpose, such as differential scanning fluorimetry (DSF), circular dichroism (CD), dynamic light scattering (DLS) and enzymatic activity assays.

Keywords: Ionic Liquids; Drug Delivery Systems; Phenylalanine Hydroxylase; Interactions; Therapeutic Proteins.

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P35. Cellular and Functional characterization of Type 2 NADH:quinone oxidoreductases from *Staphylococcus aureus*

[1]*Sena, F.V., [1]Sousa, F.M., [1]Pereira, A.R., [1]Pinho. M.G., [1,2]Pereira, M.M.

[1]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. Da República EAN, 2780-157 Oeiras, Portugal*
[2]*University of Lisbon, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Campo Grande, C8, 1749-016 Lisboa, Portugal*
lead presenter: fsena@itqb.unl.pt

NDH-2s are membrane proteins involved in respiratory chains, recognized as suitable targets for novel drug therapies. They are the only enzymes with NADH:quinone oxidoreductase activity expressed in many pathogenic organisms, as *Staphylococcus aureus*, a worldwide problem in clinical medicine. *S. aureus* presents two genes encoding NDH-2s (NDH-2A and NDH-2B) and no genes coding for Complex I, the canonical respiratory NADH:quinone oxidoreductase. This genomic information raises many questions regarding the function of NDH-2A and NDH-2B in *S. aureus*: whether the two proteins perform the same function, where they are localized, or if they are essential for *S.aureus* growth. In this study, additionally to a thorough biochemical characterization of NDH-2B, we created knockout mutants for both NDH-2s to assess their role in *S. aureus* metabolism. Growth fitness and NMR-based metabolomics were used to study the generated mutants. We also explored the spatial-temporal organization of the enzymes in cell context by fluorescence microscopy using mNeonGreen-labelled proteins. We used Super-Resolution microscopy to analyze the dynamics of cell shape and size during the cell cycle of *S. aureus* strains. Our results suggest NDH-2B works as a NADPH:quinone oxidoreductase. The ΔNDH-2B strain showed a similar growth profile to that of the wild-type strain, but cells presented larger volumes during division. NDH-2A localized both in foci at the cell membrane and in the cytoplasm. The ΔNDH-2A strain showed a slower growth and seems to have a longer phase 3 of cell cycle meaning that septal synthesis or cell splitting may be impaired in this mutant. NMR-based metabolomics showed all the strains were able to catabolize glucose and interestingly none of the strains is capable of catabolize acetate. The ΔNDH-2A strain showed higher extracellular levels of lactate. These results suggest that the two enzymes do not perform the same function, being both relevant for the metabolism of *S. aureus*.

Keywords: Staphylococcus aureus; metabolism; NDH-2; NADH/NAPH; cell cycle.

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P36. Detection and quantification of antibiotic resistance genes along the wastewater treatment process: optimization of molecular techniques

[1,2]*Oliveira, M., [2]Nunes, M., [3]Silva, A.F. & [1,2]Barreto Crespo, M.T.

[1]ITQB, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa; Av. da República, 2780-157 Oeiras, Portugal
[2]iBET, Instituto de Biologia Experimental e Tecnológica; Apartado 12, 2780-901 Oeiras, Portugal
[3]Section of Microbiology, Department of Biology, University of Copenhagen, Denmark; Nørregade 10, 1165 København, Denmark
**lead presenter: micaelaoliveira@itqb.unl.pt*

Water scarcity is a worldwide problem. Agriculture alone accounts for 70% consumption of the available fresh water on Earth[1]. This scenario makes wastewater reuse for agriculture irrigation a valuable and sustainable alternative. However, the safety of this practice is not yet assured, since the conventionally applied treatments in the wastewater treatment plants (WWTPs) appear to be inefficient towards the removal of some emerging pollutants, such as antibiotics (AB), AB resistant bacteria (ARB) and AB resistance genes (ARG)[2,3]. Carbapenems are an important group of last-line β-lactam antibiotics, only used in the hospital setting, to which ARB and corresponding genes are starting to be detected in the wastewater environment, before and after the treatments applied in the WWTPs[4]. In addition, it is common to observe simultaneous resistance to quinolones, a group of widely used antibiotics, in both hospital and outpatient settings[5]. Therefore, the first goal of this study is the development and optimization of molecular techniques that will allow an accurate evaluation of the presence of those ARB and corresponding genes in wastewater. For that, the strategy is: 1) to develop and optimize *TaqMan* multiplex qPCR protocols for the identification and quantification of 5 carbapenem resistance genes (*bla*KPC, *bla*OXA-48, *bla*NDM, *bla*IMP and *bla*VIM) and 3 quinolone resistance genes (*qnr*A, *qnr*B and *qnr*S) at different points of the wastewater treatment train; 2) to optimize the combination of propidium monoazide (which can differentiate between live and dead cells) with qPCR, in order to evaluate the risk associated with the presence of living ARB before and after the conventional wastewater treatments. It is expected that these protocols will make it possible to assess the efficiency of the currently applied wastewater treatments on the removal of these pollutants and, ultimately, help clarifying the role of WWTPs on AB resistance proliferation.

Keywords: Wastewater reuse; Antibiotic resistant bacteria; Carbapenem and quinolone resistance genes; *TaqMan* multiplex qPCR.

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List of presenting authors

Author	Email	Oral presentation
Eleonora Marini	eleonora@itqb.unl.pt	O1
Pedro Fernandes	pedro.fernandes@itqb.unl.pt	O2
Aristides Mendes	al.mendes@itqb.unl.pt	O3
Inês Torcato	inestorcato@itqb.unl.pt	O4
Amaury Fasquelle	amaury@itqb.unl.pt	O5
Cátia Bárria	catiabarria@itqb.unl.pt	O6
José Salvado	jsalvado@itqb.unl.pt	O7
Ricardo Santos	ricardo.santos@itqb.unl.pt	O8
Rita Borba	borba@itqb.unl.pt	O9
Celso Martins	celso@itqb.unl.pt	O10
Catarina Florindo	catarina.florindo@itqb.unl.pt	O11
Maria Assunção	massuncao@itqb.unl.pt	O12
Joana Rodrigues	joana.rar@itqb.unl.pt	O13
Tiago Jorge	tiagoj@itqb.unl.pt	O14
Maria Niño-González	mfgonzalez@igc.gulbenkian.pt	O15
Hélia Sales	helia.sales@itqb.unl.pt	O16
Ana Leal	ana.leal@itqb.unl.pt	O17
Sara Colanero	sara s.colanero@santannapisa.it	O18
Francisco Leisico	franciscoleisico@fct.unl.pt	O19
Bárbara Abreu	barbara.abreu@itqb.unl.pt	O20
Filipa Calisto	fcalisto@itqb.unl.pt	O21
Viviana Correia	vivianacorreia@campus.fct.unl.pt	O22
Davide Cruz	davide@itqb.unl.pt	O23
Sónia Zacarias	sonia.zacarias@itqb.unl.pt	O24
Nicole Vieira	nicole.morgado@itqb.unl.pt	O25
Ana Fernandes	ana.fernandes@itqb.unl.pt	O26
Agostinho Alexandre	agostinho@itqb.unl.pt	O27
Mara Pinto	mara.pinto@itqb.unl.pt	O28
Raquel Santos	raquel.a.santos@ist.utl.pt	O29
Marcos Sousa	msousa@ibet.pt	O30
Ana Pádua	ac.padua@campus.fct.unl.pt	O31
Diego Borges	borges.biomed@gmail.com	O32
Pedro Pedrosa	pedrompedrosa@gmail.com	O33
Author	**Email**	**Poster Presentation**
Pedro Carvalho	pedro.carvalho@itqb.unl.pt	P1
Liliana Silva	lsilva@itqb.unl.pt	P2
Andreia Silva	andreiasilva@ibet.pt	P3
Micael Silva	msi.silva@campus.fct.unl.pt	P4
Fredilson Melo	fmelo@itqb.unl.pt	P5
Stefano Nones	stefano.nones@itqb.unl.pt	P6
Rui Martins	ruifrmamartins@gmail.com	P7

Ana Rita Oliveira	ana.rpo@itqb.unl.pt	P8
Rita Ramos	rramos@itqb.unl.pt	P9
Maria Mendes	mtmendes@itqb.unl.pt	P10
Delfim Ferreira	dferreira@itqb.unl.pt	P11
Filipa Lopes	filipa.lara.fl@gmail.com	P12
Davide Martins	dcmartins@itqb.unl.pt	P13
Letice Gonçalves	leticeg@itqb.unl.pt	P14
José Rodrigues	jose.rodrigues@itqb.unl.pt	P15
Filipe Sousa	filipe.sousa@itqb.unl.pt	P16
Carolina Cordeiro	carolinacordeiro@itqb.unl.pt	P17
Dizimalta Miquitaio	dizimalta@itqb.unl.pt	P18
Carlos Moreira	carlosmoreira@itqb.unl.pt	P19
Sara Tedesco	sara.tedesco@itqb.unl.pt	P20
Diana Silva	dsilva@itqb.unl.pt	P21
Bruno Peixoto	bpeixoto@itqb.unl.pt	P22
Ricardo Vilela	rvilela@itqb.unl.pt	P23
Liliana Rodrigues	liliana.rodrigues@itqb.unl.pt	P24
Joana Bugalhão	joana.bugalhao@gmail.com	P25
Inês Trindade	ines.trindade@itqb.unl.pt	P26
Vanda Póvoa	vanda.povoa@neuro.fchampalimaud.org	P27
Maria Moita	mraquelmoita@gmail.com	P28
Valéria Custódio	valeria.custodio@itqb.unl.pt	P29
Bruno Saraiva	bsaraiva@itqb.unl.pt	P30
Anabel Rodriguez	anabel.rodriguez@neuro.fchampalimaud.org	P31
Patrícia Zoio	patricia.zoio@itqb.unl.pt	P32
Sara Handem	sarahandem@itqb.unl.pt	P33
Márcia Alves	marcia.alves@itqb.unl.pt	P34
Filipa Sena	fsena@itqb.unl.pt	P35
Micaela Oliveira	micaelaoliveira@ibet.pt	P36