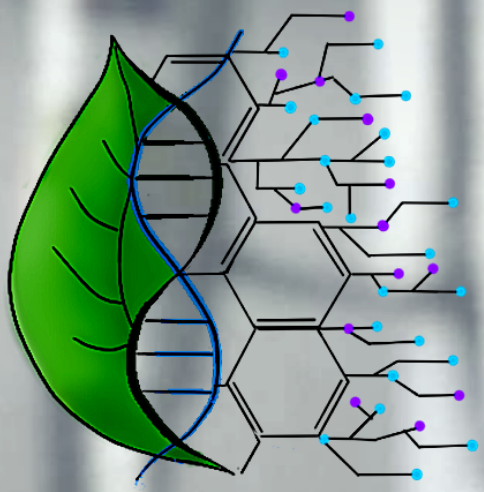


II Symposium on Applied Biochemistry

2021

BOOK OF ABSTRACTS

Universidade do Minho
Escola de Ciências

ISBN| 978-989-98830-1-7

DOI | 10.21814/1822.70576

Editor / ***Editor*** | Universidade do Minho, Departamento de Biologia, Escola de Ciências

Autor / ***Authors*** |Diana Pereira

Co-autor (es) / ***Co-author(s)*** |Björn Johansson, Inês Caldeira, Maria João Sousa, Paula Margarida, Sandra Paiva

Membros / ***Members*** | Ana Oliveira, Catarina Coelho, Cláudia Teixeira, Cristina Martins, Cristina Rodrigues, Diogo Ferreira, João Carlos Marcos, Inês Caldeira, Liliana Araújo, Paulo Mendonça, Pedro Fernandes, Rosana Alves, Sílvia Lima, Vânia Dourado

Coordenação / ***Coordination*** |Chair and Co-Chairs of the Organizing Committee of the II Symposium on Applied Biochemistry

Distribuição / ***Distribution*** | Department of Biology
University of Minho
Campus Gualtar
4710-057 Braga (Portugal)

Email: iisimposiodebioquimicaaplicada@gmail.com

URL: https://iisimposioembqa.wixsite.com/iisimposioembqa

Capa / ***Cover*** | Pedro Fernandes, Liliana Araújo

Universidade do Minho
Escola de Ciências

II SIMPÓSIO EM BIOQUÍMICA APLICADA

29.JANEIRO.2021

Evento online!

Inscrições gratuitas em:
https://iisimposioembqa.wixsite.com/iisimposioembqa

SCAN ME

Organização:

Alunos e Comissão de Curso do Mestrado em Bioquímica Aplicada

iisimposioembioquimicaaplicada

@mbqaum

iisimposiodebioquimicaaplicada@gmail.com

Index

Program

Opening session

9h-9h30	**Rui Vieira de Castro -** Dean of the University of Minho **Manuela Côrte-Real –** President of the School of Sciences, University of Minho **Maria João Sousa –** Director of the Department of Biology, University of Minho **Ana Paula Esteves –** Director of Department of Chemistry, University of Minho **Sandra Paiva –** Director of the Master Course in Applied Biochemistry **Diana Pereira -** *Chair* students of the Organization Committee

Session 1- Research in Biochemistry

9h30-10h	**Maria João Amorim –** Gulbenkian Institute of Science New paradigms in cell biology and their implications for influenza A virus lifecycle
10h-10h30	**Isabel Cardoso –** Health Research and Innovation Institute Unveiling the mechanisms of Transthyretin neuroprotection in Alzheimer's Disease: from knowledge to therapy
10h30-10h45	**Coffee Break**
10h45-11h	**Flash Talks - *Alumni*** **Sofia Oliveira-Pinto –** University of Minho Characterization of the activity of novel chromene derivatives for cancer therapy
11h-11h15	**Flash Talks - *Alumni*** **Sara Pereira –** University of Minho Obesity-related transcripts abundance in human spermatozoa influence the outcome of medical-assisted reproduction treatments
11h15-11h45	**Pedro Oliveira –** University of Aveiro Dietary habits and endocrine control of testicular cells metabolic cooperation modulate fertility
12h-14h	**Lunch**
14h-14h30	**Paula Sampaio –** University of Minho Development of a vaccine against systemic candidiasis infections

Session 2- Teaching in Biochemistry

14h45-15h	**A teaching project in Biochemistry** *De novo* plasmids constructions without commercial kits by *In-vivo* homologous recombination, for applied biochemistry applications

Session 3- Best Abstract Awards

15h-15h10	**Tomás Werner –** University of Minho Studying the evolution of gene networks through *Marchantia polymorpha*
15h10-15h20	**Catarina Medeiros –** CITAB, University of Trás-os-Montes and Alto Douro Morphometric measurements in HPV-transgenic mice after topical application of *Cytinus hypocistis (L.)* extract
15h20-15h30	**Paulo César Silva –** CBMA, University of Minho A novel D-xylose isomerase: from the gut of a wood feeding beetle for improved conversion in *Saccharomyces cerevisiae*

15h30-15h45 **Coffee Break**

Session 4-Projects between Academia and Industry

15h45-16h **Maria João Sousa –** University of Miinho

Omics approaches towards the industrial exploitation of Torulaspora delbrueckii: Elucidation of the molecular basis underlying complex cellular traits

16h-16h15 **Björn Johansson –** University of Minho

FATVAL – Systematic metabolic engineering of *Saccharomyces cerevisiae* for high-value fatty acids and derived products

16h15-16h30 **Carmen Becerra**

Yeast Doc – Studying yeast metabolism for wine industry

Session 5-Biochemistry in Industry

16h30-17h **Ana Loureiro –** BIAL

Discovery and development of Etamicastat, a dopamine beta-hydroxylase inhibitor

17h-17h30 **Pedro Castanheira –** IMMUNETHEP

Between Academy and Industry

17h30-18h20 **Ana Loureiro & Pedro Castanheira**

Career Development Session

18h20-18h25 **Coffee Break**

18h25-18h40 **Flash Talks - *Alumni***

Rosana Alves – University of Minho

A Biochemistry career path: from Bachelor to PhD

Closing session

18h40 **Sandra Paiva –** Director of the Master Course in Applied Biochemistry

Diana Pereira - *Chair* students of the Organization Committee

Inês Caldeira - Co-Chair students of the organizing Committee

Welcome message

Dear participants,

The II Symposium in Applied Biochemistry was held online on January 29th 2021, with over 250 participants. The main objectives of the Symposium were to promote the discussion of recent scientific advances in the field of Biochemistry and to inspire future students towards the Master (MSc) course in Applied Biochemistry, of the University of Minho. Despite the unique circumstances experienced during this period, the event proved to be a success and exceeded our best expectations.

The Symposium was organized into three main sessions: Research in Biochemistry, Biochemistry in Industry and Career Development. It also included Flash Talk presentations from former students of the MSc course in Applied Biochemistry and sessions dedicated to Teaching in Biochemistry and to presentations of Projects between Academia and Industry. Additionally, three abstracts, out of the 18 accepted by the Scientific Committee, were chosen for oral presentations, and were selected as "Best Abstract Awards". The Interaction between participants and researchers was possible after each session and different career paths were on display.

The Symposium received significant media coverage and we are very happy that all the stated goals were fulfilled and that a pleasant and exciting moment for scientific exchange was provided.

Thank you very much to all the participants and speakers who contributed to the success of the II Symposium on Applied Biochemistry.

We hope to see you in the III Symposium on Applied Biochemistry!

The Organizing Committee of the II Symposium on Applied Biochemistry

Webpage: https://iisimposioembqa.wixsite.com/iisimposioembqa
Facebook: https://www.facebook.com/iisimposiobqaplicada

Organization

Scientific and Organizing Committee

Sandra Paiva

Director of the Master Course (MSc) in Applied Biochemistry - Chair Teachers

Paula Margarida Ferreira

Member of the Course Committee of MSc in Applied Biochemistry

Björn Johansson

Member of the Course Committee of MSc in Applied Biochemistry

Maria João Sousa

Director of the Department of Biology

Sílvia Lima

Director of the Degree Couse in Biochemistry

João Carlos Marcos

Professor of the Chemistry Department

Rosana Alves

Professor of the Biology Department

Organizing Committee

Students of the MSc in Applied Biochemistry - 2nd Year

Diana Pereira
Chair students

Alexandre Mendonça
Marketing

Inês Caldeira
Co-Chair students

Catarina Coelho
Marketing

Cláudia Teixeira
Treasury

Cristina Martins
Marketing

Pedro Fernandes
Media

Vânia Dourado
Marketing

Cristina Rodrigues
Media

Diogo Ferreira
Logistics

Liliana Araújo
Media

Clara Oliveira
Logistics

Lectures

Session 1-Research in Biochemistry

New paradigms in cell biology and their implications for influenza A virus lifecycle

Maria João Amorim

Laboratory of Cell Biology of Viral Infection, Gulbenkian Institute of Science, Lisbon.

Viruses impose serious challenges to public health. Being intracellular parasites with a limited repertoire of proteins, viruses need to use cellular resources and pathways for efficient replication. Viruses can simply use host pathways but can also modify them, or alternatively use the cell's resources to build their own organelles. Since many of these processes are specific to viruses and do not normally occur, understanding them not only brings knowledge about the virus but can also give rise to new antiviral therapies. Traditionally, organelles are seen as structures bounded by membranes, a fact that allows them to retain and be permeable only to selected molecules. However, in the cell other structures are known, such as the nucleolus, stress granules, centrosomes, which form individualized compartments, with a unique composition, but which are not delimited by membranes. Recently, it was discovered that these membraneless organelles are formed by a liquid-liquid phase separation mechanism, similar to what happens when we mix oil and vinegar. My laboratory has dedicated to studying how the influenza A virus, an important pathogen that causes recurrent annual and pandemic epidemics, takes advantage of this novel discovered mechanism to organize biochemical reactions to benefit its replication. We found out that the influenza A virus builds membraneless organelles by separating them into phases in order to assemble its genome. This is very important, because the influenza A virus is segmented, formed by eight different RNA segments by a selective mechanism, of which little is still known. Understanding this process will make it possible to better fight epidemics and pandemics caused by influenza A virus.

Unveiling the mechanisms of Transthyretin neuroprotection in Alzheimer's Disease: from knowledge to therapy

Isabel Cardoso

Molecular Neurobiology, I3S, Porto

Alzheimer's Disease (AD), the most prevalent form of dementia, is predicted to affect over 100 million all over the world by 2050. To date, there are no approved disease-modifying drugs for AD. New approaches are urgently needed.

AD results from Aβ peptide accumulation in the brain, due to an imbalance between its production and elimination. Aβ triggers many of the important events described in AD, including synaptic and neuronal injury and cell death. Structural vascular alterations also occur in AD, such as thickening of the basement membrane (BM) of brain vessels, speculated to function as a physical barrier to Aβ clearance across the blood-brain barrier (BBB).

Transthyretin (TTR) is an homotetrameric protein synthesized by the liver and the choroid plexus, and secreted to blood and cerebrospinal fluid (CSF), respectively. TTR represents 20% of the total CSF proteins. The importance of TTR in neuroprotection is robustly established in AD pathogenesis by us and others, both preclinically and clinically: TTR levels are decreased in CSF and plasma of AD patients; AD mice with genetic TTR reduction (AD/TTR+/-) show increased Aβ deposition, compared to AD/TTR+/+, whereas overexpressing human TTR in an AD mouse model decreases Aβ deposition. Several *in vitro* studies by us and others, suggest that TTR binds Aβ, avoiding its aggregation and toxicity. Additionally, we showed that TTR promotes Aβ brain efflux through the BBB. Recently, we demonstrated that TTR also regulates the vascular function: The BM is thicker in mice with TTR reduction, both AD and non-transgenic mice. Also, our preliminary *in vivo* studies suggest that TTR is angiogenic.

Of note, we showed that TTR is unstable in AD. TTR stabilization by tetrameric stabilizers, such as iododiflunisal, recovers its activity and improves the AD-like pathology in AD mice. Our consortium carried out a project in which we identified, from thousands of compounds selected by virtual screening and by various biological tests, the 10 best TTR stabilizers. Among them, 4 molecules are compounds approved for other pathologies and that can be repositioned for AD, once their beneficial effect is demonstrated in

preclinical trials, with AD mice. Thus, we propose a radically innovative approach to AD therapeutics: the stabilization of TTR using small molecule stabilizers, in order to restore its blood/CSF levels and activity.

Dietary habits and endocrine control of testicular cells metabolic cooperation modulate fertility

Pedro Oliveira

Department of Chemistry, University of Aveiro

Male fertility issues have been overlooked for many years. Although statistics from the World Health Organization (WHO) clearly show that sperm quality is decreasing worldwide to dangerous levels that can compromise natural conception, the molecular mechanisms that control spermatogenesis remain as a technically challenging topic. Spermatogenesis is a complex and highly regulated process orchestrated by testicular cells. In the 19th century, Enrico Sertoli described for the first time irregularly shaped somatic cells in the testis and suggested that they do not produce spermatozoa but support their production. Indeed, those cells form the blood-testis barrier. This way, the Sertoli cells (SCs) separate the interstitial fluid from the intratubular fluid. Later it was shown that SCs not only control the passage of substances and metabolites for the compartment where germ cells develop by mechanisms sensitive to hormones, but also establish a strict metabolic cooperation with developing germ cells. The distinct energetic needs during the different stages of spermatogenesis are controlled by SCs that produce the lactate used as main energy source by developing germ cells. This topic has been overlooked even though there is an increase on the prevalence of metabolic diseases that dysregulate systemic metabolism and may disrupt this testicular cells metabolic cooperation. Most studies aiming to correlate the incidence of metabolic diseases with sperm quality failed to show a clear causal relationship and thus there is not yet a consensus. Recently, several authors showed the impact of hormonal dysfunction and metabolite shifting caused by those diseases in the testis, particularly on the metabolic cooperation established between testicular cells. Thus, it is crucial to understand how those mechanisms are sensitive to energy homeostasis regulating hormones and dietary habits. Overall, the study of SCs is an emerging field for researchers interested in the understanding of the molecular mechanisms responsible for male (in)fertility. Ultimately, those studies may highlight new therapeutic targets for the control of male fertility.

Development of a vaccine against systemic candidiasis infections

Paula Sampaio

Department of Biology, University of Minho, Braga

Systemic fungal infections have been increasing significantly in recent decades, particularly in the population of immunocompromised individuals. The treatment of these infections is limited to a reduced set of anti-fungal agents which, in addition to adverse side effects, has reduced efficacy due to increased resistance in strains. In the Micro II research group, we are developing a vaccine to prevent fungal infections, including systemic candidiasis and protect the most susceptible groups of the population. This seminar will address the problematic issue of nosocomial fungal infections, the most common methods of treatment, the concerns with vaccination of commensal organisms and the work developed by our research group towards the development of a vaccine against *Candida* infections.

Flash Talks - *Alumni*

Characterization of the activity of novel chromene derivatives for cancer therapy

Sofia Oliveira-Pinto[1,2,a], Olívia Pontes[1,2,a], Diogo Lopes[3,a], Belém Sampaio-Marques[1,2], Marta D. Costa[1,2], Luísa Carvalho[1,2], Céline S. Gonçalves[1,2], Bruno M. Costa[1,2], Patrícia Maciel[1,2], Paula Ludovico[1,2], Fernanda Proença[3], Fátima Baltazar[1,2] and Marta Costa[1,2]

[1] Life and Health Sciences Research Institute (ICVS), University of Minho, Campus of Gualtar, Braga, Portugal

[2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

[3] Department of Chemistry, University of Minho, Campus of Gualtar, Braga, Portugal

[a] These authors contributed equally to this work.

Contact for the abstract: Sofia Oliveira-Pinto sofspinto.bio@gmail.com

Breast cancer (BC) is the second most diagnosed cancer worldwide and the main cause of cancer-related death in women. The current therapeutic options to treat these patients are not sufficiently effective and present several limitations. Thus, the search for new anti-BC agents is of urgent demand. Chromenes are privileged scaffolds in medicinal chemistry and therefore considered promising anticancer drugs.

The potential of the synthetized chromenes to decrease cell viability and to inhibit several cancer aggressiveness features were assessed using different BC cell lines (MCF-7, Hs578t and MDA-MB-231). *In vivo* toxicity was also investigated using the *C. elegans* model.

The results suggest that this family of compounds has a promising anticancer profile, with enhanced activity for the luminal cancer subtype (MCF-7). The newly synthesized compounds presented higher cytotoxicity for malignant cells than for non-neoplastic cells (MCF-10A), in general with good selectivity index. The most promising compounds were able to inhibit cell proliferation, induce cell cycle arrest, apoptosis, and microtubule destabilization for MCF-7 cell line. These compounds were also evaluated for their *in vivo* toxicity profile and no adverse effect was detected.

In general, the results suggest that these new compounds are promising candidates for the treatment of this type of cancer, demonstrating a high activity and specificity.

Keywords:

Anticancer activity, Breast cancer, Chromenes, *C. elegans*

Obesity-related transcripts abundance in human spermatozoa influence the outcome of medical-assisted reproduction treatments

Sara C. Pereira[1], Ana D. Martins[1,5], Mariana P. Monteiro[1], Soraia Pinto[2], Alberto Barros[2,3,4], Pedro F. Oliveira[5], Marco G. Alves[1]

[1]Department of Anatomy, Endocrine, Cardiovascular & Metabolic Research, Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal

[2]Centre for Reproductive Genetics Professor Alberto Barros, Porto, Portugal

[3]Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal

[4]i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

[5]QOPNA & LAQV, Department of Chemistry, University of Aveiro, Portugal.

Contact for the abstract: Marco G. Alves alvesmarc@gmail.com

As a haploid cell, sperm has highly condensed DNA, ensuring a stable conformation and safe delivery to the oocyte. This conformation leads to the cessation of (almost) all translational activity. Nevertheless, mature spermatozoa carry a pool of mRNAs, which function, and origin remains unknown. In this work, we propose to study whether the abundance of obesity-related gene (ORG) transcripts in human sperm is related to the fertility potential of men. To test our hypothesis, the abundance of three specific obesity-related transcripts, melanocortin-4 receptor *(MC4R),* glucosamine-6-phosphate deaminase 2 (*GNPDA2),* and fat mass and obesity gene (*FTO*), was evaluated by the quantitative polymerase chain reaction (qPCR), and several clinical data were collected from 106 couples undergoing medical-assisted reproduction treatments (ART).

Our results reveal that the ORG transcripts abundance in human spermatozoa is correlated with various parameters of fertility potential. The *MC4R* transcript abundance in human spermatozoa is negatively correlated with the percentage of viable sperm in the ejaculate (r = -0.3111) and positively correlated (r = 0.4420) with pregnancy, through the hormone levels of human chorionic beta gonadotropin. The *FTO* transcript abundance in human spermatozoa is positively correlated with the total number of sperm in the ejaculate (r = 0.5030), and also with the indicators of embryonic development, namely the rate fertilization rate (r = 0.4885), embryo cleavage rate (r = 0.5705) and high-quality embryo rate (r = 0.6998). We could not find any correlation between the *GNPDA2* transcript abundance in human spermatozoa and any of the fertility parameters studied.

We concluded that the abundance of ORG transcripts in human spermatozoa has the potential to affect the outcome of ART, through both sperm quality, with pre-implantation embryo development and pregnancy. Our results highlight the importance of monitoring paternal health during natural conception and ART.

Keywords:
Obesity-related genes; spermatozoa; assisted reproduction; MC4R; FTO

A Biochemistry career path: from Bachelor to PhD

Rosana Alves[1,2]

[1] Department of Biology, School of Sciences, University of Minho, Braga, Portugal

[2] Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal

Contact for the abstract: Rosana Alves rosana@bio.uminho.pt

Rosana Alves holds a BSc in Biochemistry from the University of Porto (2011), a MSc in Applied Biochemistry from the University of Minho (2013) and a PhD in Molecular Biology, with a specialization in Cell Biology and Health, also from the University of Minho (2020). She is currently an Invited Professor in the Department of Biology and a Postdoc Researcher in the Centre of Molecular and Environmental Biology at the University of Minho.

This talk will highlight her research path in Biochemistry, developed both in Portugal and abroad, involving the study of carboxylate transporters in *Candida* species. These are the most frequent opportunistic yeasts in humans and a common source of systemic infections in hospitalized patients. The urgent need for novel therapies to treat these infections has risen the efforts to unravel the genetic circuits governing immune evasion, host adaptation, biofilm formation and antifungal drug resistance.

Keywords:

Candida species**,** carboxylate transporters, biofilms, immune evasion, antifungal drug resistance

Session 2- Teaching in Biochemistry

De novo plasmids constructions without commercial kits by *In-vivo* homologous recombination, for applied biochemistry applications

Cíntia Mendes[1], Lavínia Pinto[1], Diana Ribeiro[1], Rita Amaral[1], Inês Abreu[1], Leandra Armada[1], Bruna Ortiga[1], Margarida Costa[1], Sophia Lorenz[1], Juliana Thomaz[1], Maria João Fernandes Moreira[1], Maria Gabriela Gonçalves[1], Tatiana Pozdniakova[2] and Björn Johansson[2]

[1] Undergraduate student of Biological Engineering (Integrated Master Course 9358)

[2] Department of Biology/Centre of Molecular and Environmental Biology (CBMA/MEC), University of Minho, Braga, Portugal

The construction of a new plasmid is a project that can practically be carried out during an undergraduate laboratory course. Plasmid construction can also be a valuable pedagogical tool as it involves many fundamental techniques. It also represents a real-life example of work often performed in a research laboratory.

Plasmids are small DNA molecules that are physically separate from the genomic DNA and can replicate independently. The most common form of these, is a circular double-stranded DNA molecule in bacteria, archaea, and some eukaryotic organisms such as baker's yeast *Saccharomyces cerevisiae*. They usually carry genes that are responsible for useful features, for example, the resistance to certain antibiotics.

Commercial kits and enzymes are often used for DNA isolation, purification and to cut and ligate DNA molecules, but are prohibitively expensive to use in a teaching course with many participants. The students of the 2020 edition of "*Laboratórios Integrados de Biologia 9503N6*" used a combination of protocols that relied exclusively on in-house prepared reagents, without commercial kits. Briefly, we obtained DNA fragments by PCR amplification of plasmids from previously selected *Escherichia coli* cultures. We generated new plasmids that contained different selection markers and origins of replication by *in-vivo* homologous recombination. Homologous recombination depends on the DNA repair machinery of the cell to join DNA fragments that share short stretches of identical sequences.

We obtained four new distinct plasmids, pTA5, pTA6, pTA8 and pTA10, that can be used to express genes for heterologous proteins or entire metabolic pathways of interest. The resulting plasmids will be used in the FatVal research project for the expression of metabolic pathways to produce specialized fatty acids, as well as pathways facilitating the metabolism of the pentose sugar D-xylose.

In conclusion, the students discovered how to work with DNA in the laboratory through the experiments. The contact with a real-life research project was pedagogically enriching from a future professional life perspective.

Acknowledgements:

This work was supported by the project FatVal PTDC/EAM-AMB/32506/2017 (POCI-01-0145-FEDER-032506), co-funded by the European Regional Development Fund (ERDF), through the Operational Programme for Competitiveness and Internationalization (COMPETE 2020), under Portugal 2020, and by the Fundação para a Ciência e a Tecnologia – FCT I.P through national funds.

Session 3-Best Abstract Awards

Studying the evolution of gene networks through *Marchantia polymorpha*

Tomás Werner, Sara Coelho, Sara Laranjeira, Rómulo Sobral, M. Manuela R. Costa

BioISI- Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho,Campus de Gualtar, 4710-057 Braga, Portugal
Corresponding author: Tomás Werner (tomas@abreuwerner.com)

The establishment of new interactions within a gene regulatory network drives the emergence of new biological functions and morphologies. New interactions are often associated with repeated events of gene duplication that increase the genome complexity. Exploring the plant evolutionary path and understanding the function of a given gene is thus challenging due to redundancy within multigene families. The analysis of gene function in early land plants allows the study of ancestral gene functions, due to the existence of fewer duplicated gene copies, and enables a better understanding of the establishment of gene regulatory networks. The DDR transcriptional module is composed by three MYB proteins- DIVARICATA (DIV), RADIALIS (RAD) and DIV-and-RAD-Interacting Factors (DRIF) - and it controls several development processes in angiosperms, such as flower asymmetry in Antirrhinum majus and fruit development in tomato. To elucidate early functions of these proteins, we are studying DIV and DRIF homologs of Marchantia polymorpha, a liverwort that is used as a basal plant model. To unravel how the DDR module was first established, transgenic plants with gene function knocked out by the CRISPR/Cas9 editing system, with promoter::reporter gene fusions and with ubiquitous expression have been generated and their phenotypes are being studied. The results of this study could yield a better understanding of the evolution of gene regulatory networks and of how this module functions.

References:

-Coelho, S. (2019). Study of ancestral genes in Marchantia polymorpha (MSc). Universidade do Minho.
-Raimundo, J., Sobral, R., Laranjeira, S., & Costa, M. M. R. (2018). Successive domain rearrangements underlie the evolution of a regulatory module controlled by a small interfering peptide. Molecular Biology and Evolution, 35(12). https://doi.org/10.1093/molbev/msy178
-Raimundo, J., Sobral, R., Bailey, P., Azevedo, H., Galego, L., Almeida, J., ... Costa, M. M. R. (2013). A subcellular tug of war involving three MYB-like proteins underlies a molecular antagonism in Antirrhinum flower asymmetry. The Plant Journal, 75(4), 527–538. https://doi.org/10.1111/tpj.12225

Acknowledgements

This work was supported by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior through national funds (Programa de Investimento e Despesas de Desenvolvimento da Administração Central) with the project grant PTDC/BIA-PLA/1402/2014 and by a Research Unit grant UID/MULTI/04046/2019 to BioISI.

Morphometric measurements in HPV-transgenic mice after topical application of *Cytinus hypocistis* (L.) extract

C. Medeiros[1*], A. R. Silva [2,3*], T. Ferreira[3], L. Barros[2], I.C.F.R. Ferreira[2], M.J. Pires[1], R. Gil da Costa[1], A. Gama[4], P.A. Oliveira [1#]

[1] CITAB, UTAD, Vila Real, Portugal;

[2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal;

[3]Departamento de Ciências Farmacêuticas, Faculdade de Farmácia, CIETUS-IBSAL, Universidade de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Espanha;

[4]CECAV, UTAD, Vila Real, Portugal;

*Both authors contributed equally to this work.

#pamo@utad.pt

Human papillomavirus (HPV) infections are associated with the development of several anogenital cancers. K14HPV16 transgenic model is an animal model that mimics HPV-induced lesions. *Cytinus hypocistis* (L.) is an endophytic parasitic plant that has been shown to possess several antibacterial and antiproliferative properties1. During antiproliferative studies in animal models, the toxicological analysis is also required. Therefore, the present research aims to study the toxic effects of applying a topical formulation enriched with *Cytinus hypocistis* extracts (FCH) on K14HPV16 mice.

Three different concentrations of FCH were used to enrich a base cream, the phenolic compounds were analyzed by HPLC-DAD-ESI/MS and were applied to the animal's ears for 28 days. Thirty female mice were equally divided into six groups (G) (n=5): G1 (HPV16+-C1); G2 (HPV16+-C2); G3 (HPV16+-C3); G4 (HPV-C3); G5 (HPV+-control); and G6 (HPV-control). The animals were kept under controlled conditions and biological variables were registered throughout the study. In the end, animals were sacrificed, and the organs collected and weighed.

The main phenolic compound present in the base cream enriched with *C. hypocistis* was galloyl-bis-HHDP-glucose. Food and water intake were constant throughout the trial. G3 presented a higher relative weight of the liver and lungs than the control animals, and a significant difference in body weight relatively to G4 ($p<0.05$). The body weight loss and the increase in liver weight observed in HPV+ mice treated with they may reflect a negative impact of the extract on these already fragile animals. However, overall, the FCH was well tolerated by the animals.

Although the highest concentration of the extract had a negative impact on the evaluated morphometric parameters, this does not necessarily imply that the extract is toxic, which can be corroborated with histological analysis. Further studies are needed to understand whether different doses of this extract would cause distinct effects.

References:

1. Silva AR, *et al.*,(2020). Food and Chemical Toxicology, 136, 111071.

Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). A. R. Silva is grateful to FCT for her Doctoral Grant (SFRH/BD/145834/2019) and L. Barros for her contract through the institutional scientific employment program.

A novel D-xylose isomerase: from the gut of a wood feeding beetle for improved conversion in *Saccharomyces cerevisiae*

Paulo César Silva[a], Javier A. Ceja-Navarro[bc], Flávio Azevedo[a], Ulas Karaoz[d], Eoin L. Brodie[de] and Björn Johansson[a]

[a] CBMA - Center of Molecular and Environmental Biology, University of Minho, Campus de Gualtar, Braga, 4710-057, Portugal.

[b] Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

[c] Institute for Biodiversity Science and Sustainability, California Academy of Sciences, San Francisco, California, USA

[d] Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

[e] Department of Environmental Science, Policy and Management, University of California, Berkeley, California, USA.

Carbohydrate rich substrates such as lignocellulosic hydrolysates remain one of the primary sources of potentially renewable fuel and bulk chemicals. The pentose sugar D-xylose is often present in significant amounts along with hexoses. For low value/high volume products, yield is of paramount importance for process economy. *Saccharomyces cerevisiae* can acquire the ability to metabolize D-xylose through expression of heterologous D-xylose isomerase (XI). This enzyme is notoriously difficult to express in *S. cerevisiae* and only fourteen genes have been reported to be active so far. We cloned a new D-xylose isomerase derived from microorganisms in the gut of the wood-feeding beetle *Odontotaenius disjunctus*. Although somewhat homologous to the current gold-standard from *Piromyces sp*. E2, metagenome scaffold gene neighborhoods and metagenome binning identified the gene as of bacterial in origin and the host as a *Parabacteroides* sp. Expression of the new XI enzyme in *S. cerevisiae* resulted in faster aerobic growth on D-xylose than the XI from *Piromyces*. The D-xylose isomerization rate of the yeast expressing this new XI was also 72 % higher. Interestingly, increasing concentrations of xylitol (up to 8 g/L) appeared not to inhibit xylose consumption in both strains. The newly described XI displayed 2.6 times higher specific activity, 37 % higher affinity for D-xylose, and exhibited higher activity over a broader temperature range, retaining 51 % of maximal activity at 30 °C compared with only 29% activity for the *Piromyces* XI. This new enzyme represents a highly valuable addition to the *S. cerevisiae* molecular toolbox and shows promise for improved industrial conversion of carbohydrates.

Acknowledgments

This work was supported by the project FatVal PTDC/EAM-AMB/32506/2017 (POCI-01-0145-FEDER-032506), co-funded by the ERDF, through the COMPETE 2020, and by the FCT I.P. CBMA was supported

by the “Contrato-Programa” UIDB/04050/2020 funded by the FCT I.P. PCS is recipient of a FCT PhD fellowship (SFRH/BD/140039/2018).

Session 4-Projects between Academia and Industry

Omics approaches towards the industrial exploitation of *Torulaspora delbrueckii*: Elucidation of the molecular basis underlying complex cellular traits

TODOMICS – Maria João Sousa

CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Portugal.
Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho,
Portugal

Torulaspora delbrueckii is an ascomicetous yeast evolutionary close to *S. cerevisiae*, whose particular traits have caught the attention of the bread and wine industries, due to several advantageous properties when compared to the traditionally used *S. cerevisiae* yeast, such as, production of flavor and aroma-enhanced wine or the ability to be preserved for longer in frozen dough.

However, owing to limited knowledge on *T. delbrueckii*, its biotechnological exploitation is still insipient and empirical. In this context, omic sciences and the new sequencing technologies can contribute to rapidly change this scenario, fostering its exploitation. In this project, we aim to explore the expertise of the team members in the extensive study of *T. delbrueckii*, of wine and bread dough fermentations, and in genomics, to gain new insight into the biochemical and molecular bases of the specific physiological traits of this yeast. The information will be used for the genetic improvement of both *T. delbrueckii* and *S. cerevisiae*, by consumer well-accepted methods, in order to validate the phenotype/genotype relations identified and optimize both species for biotechnological application.

Acknowledgments:

This work was supported by the strategic programme UID/BIA/04050/2019 and project TODOMICS (PTDC/BIA-MIC/32059/2017) funded by national funds through the FCT, I.P. and by the ERDF through the COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and Sistema de Apoio à Investigação Científica e Tecnológica (SAICT).

FatVal: Systematic metabolic engineering of Saccharomyces cerevisiae for high value fatty acids and derived products

FatVal – Björn Johansson

Biology Department, University of Minho, Braga

Fatty acids are a class of natural compounds of potential high value and they are important parts of many classes of molecules with economical and therapeutic value. Fatty acids are currently extracted from non-sustainable sources such as fish oil which is exacerbated for rare high value fatty acids due to their very low titers in the raw material.

This project involves a systematic metabolic engineering of Saccharomyces cerevisiae strain for sustainable fatty acid production host as well as to apply the fatty acid production capabilities for a the therapeutically interesting compound, Capsaicin, through an international collaboration.

Acknowledgments:

This work was supported by the project FatVal PTDC/EAM-AMB/32506/2017 (POCI-01-0145-FEDER-032506), co-funded by the ERDF, through the COMPETE 2020, and by the FCT I.P. CBMA was supported by the "Contrato-Programa" UIDB/04050/2020 funded by the FCT I.P.

Studying yeast metabolism for wine industry

Yeast Doc – Carmen Becerra

French National Institute for Agronomic Research (INRAE), France
University of Minho, Braga, Portugal

Biochemistry and Microbiology are two highly interconnected fields with an undeniable contribution to society. Nowadays, the industry of microbial biotechnology is a clear example of that impact, comprising different areas like the pharmaceutical, biofuel or food sectors. However, the utilization of microbes for production of fermented foods and beverages is not a new event in history. Yeasts have been traditionally used for a variety of products, such as bread, cheese or beer. In my case, I work with yeasts in the context of winemaking fermentation. With my research I study the Fungal Oligopeptide Transporters (FOT) in *Saccharomyces cerevisiae*, main responsible for the consumption of oligopeptides from grape must as a source of nitrogen during fermentation. I work on the characterization of FOT using a combination of molecular biology, fermentation and bioinformatics tools. Ultimately, this work can help to better understand the role of oligopeptide transport in yeast metabolism during fermentation and the production of volatile compounds that contribute to wine aroma.

Session 5-Biochemistry in Industry

Discovery and development of Etamicastat, a dopamine beta-hydroxylase inhibitor

Ana Loureiro

BIAL Laboratories

The discovery and development of a new drug is a complex and long process that begins with an unmet medical need. After defining a therapeutic target, the synthesis of new Chemical entities (NEQs) starts. The selection is based on *in vitro* and *in vivo* tests, which evaluate the efficacy, selectivity and DMPK properties (metabolism and pharmacokinetics of drugs). Once selected, the molecule enters the pre-clinical development process where, among others, several *in vitro* and *in vivo* studies on safety pharmacology and toxicology are carried out, to guarantee the safety of the compound to be administered to humans. The decision to move to humans depends on the data collected in the non-clinical studies. During clinical studies of safety and efficacy, the possibility of failure is still quite high. The entire process of developing a drug is followed by "go-no-go" decisions. Those decisions, made at each stage, are the cornerstone of success drug development. In this presentation, the roadmap for the discovery and development of etamicastat, a dopamine beta-hydroxylase inhibitor in BIAL, will be shown. We will focus on the contribution of DMPK studies, proving its key role on the development of new molecules and therefore, new therapies.

Between Academy and Industry

Pedro Castanheira

Contact for the abstract: Pedro Castanheira pedro.castanheira@immunethep.com

IMMUNETHEP, Biocant Park - Núcleo 04 Lote 14 - 3060-197 Cantanhede - Portugal

Pedro Castanheira is a researcher with a basic training in Biochemistry. During his career he was involved in a multitude of projects ranging from the characterization of native aspartic proteases obtained from plant origin, to the recombinant expression of proteins in *Escherichia coli* with biotechnology applications or involved in neuronal diseases, such as Alzheimer's, Parkinson's, Lafora Disease or Familial transthyretin amyloidosis.

After completion of his PhD in Biochemistry, Pedro became a Researcher at Biocant, a research institute located at Biocant Park, where he interacted with multiple biotech companies located at the Park and was involved in the creation of a Biophysical Unit for the characterization of biologics. Later, moved to Immunocore in the UK, working with soluble T-Cell Receptors for the treatment of viral and bacterial infectious diseases, and more recently moved back to Portugal to join Immunethep, SA which is focused on the development of innovative immunotherapies to prevent and treat infections caused by bacteria, and also on the development of a vaccine against covid-19.

In the symposium, Pedro presented his career path and explained his vision on the transition between academia and industry.

Keywords:
biochemistry, academia, industry, biotech, startup

Accepted Abstracts

Functional analysis of AtDRIF genes during the development of Arabidopsis thaliana seedlings

Ana Marques, Rómulo Sobral, Ana Cunha, Sweta Singh, João Raimundo, Sara Laranjeira and Manuela Costa

BioISI – CBFP – University of Minho, Campus of Gualtar, 4710-057, Braga, Portugal

Plants are highly dependent on environmental signals to coordinate and synchronize their development with the most favourable conditions. Light is a major cue controlling early stages of plant development when individuals are more vulnerable to the environment. The development of plant seedlings buried in the soil (no light input, skotomorphogenesis) is characterized by the growth of the embryonic stem (hypocotyl) and the formation of an apical hook, a curvature of the stem that protects the apical meristem from soil abrasion. When the seedlings emerge from the soil, the perception of light initiates a transition characterized by a decrease in the growth rate of the hypocotyl and by the opening of the apical hook (photomorphogenesis) (Mazzella *et al.,* 2014; Wang & Guo, 2018). Many families of genetic factors are known to be involved in the transition from skoto to photomorphogenesis, however a link between them is still illusive (Kwon *et al.*, 2013). Our lab has been studying the previously unknown role of the *DIV-and-RAD-interacting-factors (DRIFs)* genes during dark to light transition. In Arabidopsis, the DRIF family is composed by five homologs, three of which (*DRIF3, DRIF4 and DRIF5*) are very similar, suggesting they may be functionally redundant (Raimundo *et al.*, 2018). The triple knockout mutant *Atdrif345* shows a shorter hypocotyl than wild type and the loss of the apical hook when grown in the dark. An RNAseq approach was used to identify genetic factors that are affected in the *Atdrif345* during early stages of development. The results suggest that *DRIF3, DRIF4 and DRIF5* are control key genes on the regulatory network that regulates the formation of the apical hook and hypocotyl elongation during the transition from skoto to photomorphogenesis. The proper development of seedlings is critical for plant fitness and crop yield, particularly in a rapidly changing environment. Thus, the study of the *DRIF* genes is important to fully understand these processes.

References:

1. Payen, V.L., Mina, E., Van Hée, V.F., Porporato, P.E., and Sonveaux, P. (2020). Monocarboxylate transporters in cancer. Mol. Metab. 33, 48–66.
2. Kwon, Y., Kim, J. H., Nguyen, H. N., Jikumaru, Y., Kamiya, Y., Hong, S.-W., & Lee, H. (2013). A novel Arabidopsis MYB-like transcription factor, MYBH, regulates hypocotyl elongation by enhancing auxin accumulation. Journal of Experimental Botany, 64(12), 3911–3922.
3. Mazzella, M. A., Casal, J. J., Muschietti, J. P., & Fox, A. R. (2014). Hormonal networks involved in apical hook development in darkness and their response to light. Frontiers in Plant Science, 5(52).
4. Raimundo, J., Sobral, R., Laranjeira, S., & Costa, M. M. R. (2018). Successive domain rearrangements underlie the evolution of a regulatory module controlled by a small interfering peptide. Molecular Biology and Evolution, 35(12).
5. Wang, Y., & Guo, H. (2018). On hormonal regulation of the dynamic apical hook development. New Phytologist, 222(3), 1230–1234.

Development of Molecular Tools for Expression and Trafficking Studies of The Human Monocarboxylate Transporters

Lopes D[1], C Barata-Antunes[1], R Alves[1], Sorkin A[2], S Paiva[1]

[1] Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal

[2] Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, USA diogomlopes96@gmail.com

Most cancer cells rely on glycolysis to sustain their high proliferation rates with the production of lactate. For many years, lactate was seen as a metabolic waste of glycolytic metabolism in the tumor microenvironment, however, lactate has been recently associated as a key metabolic fuel and as an important signaling molecule 1,2. This substrate is responsible for extracellular acidification, which, is a feature of the tumor environment, and favors tumor invasion. The transport of lactate across the plasma membrane is mediated by a family of proton coupled monocarboxylate transporters (MCTs), which comprises 14 members 3. MCT1 and MCT4 serve as metabolic links between cancer cells via lactate exchange within tumors. This form of metabolic symbiosis illustrates how the apparent waste product from hypoxic tumor cells may be exploited by oxidative tumor cells to sustain their energy production under nutrient deprived conditions 4. MCTs are not only gatekeepers of intercellular metabolic cooperation, but also important regulators of angiogenesis and tumor migration, invasion and metastasis 5 . However, the role of MCTs in tumors is far from being well understood and their potential as therapeutic targets is poorly explored. Given the relationships between MCT1 and MCT4 in cancer cells, they offer a unique opportunity for novel treatment strategies.

In this work, a set of molecular tools was generated for the expression and trafficking analyses of MCT1 and MCT4. Plasmids were designed harboring MCT1 or MCT4 with GFP or mCherry at the C- or N- terminal following the classical DNA cloning method. These molecular tools will be essential to study the expression and localization of MCT1 and MCT4 and to study the conditions and mechanisms underlying the endocytic trafficking of both transporters to further elucidate the significance of MCTs expression in tumor cells.

Keywords:

Cancer, lactate, metabolic reprograming, monocarboxylate transporters, MCT1, MCT4, molecular cloning.

References:

1. Pérez-Tomás, R., and Pérez-Guillén, I. (2020). Lactate in the Tumor Microenvironment: An Essential Molecule in Cancer Progression and Treatment. Cancers (Basel). 12.
2. de la Cruz-López, K.G., Castro-Muñoz, L.J., Reyes-Hernández, D.O., García- Carrancá, A., and Manzo-Merino, J. (2019). Lactate in the Regulation of Tumor Microenvironment and Therapeutic Approaches. Front. Oncol. 9, 1143.
3. Halestrap, A.P. (2013). Monocarboxylic Acid Transport. In Comprehensive Physiology (American Cancer Society), pp. 1611–1643.
4. Pérez-Escuredo, J., Van Hée, V.F., Sboarina, M., Falces, J., Payen, V.L., Pellerin, L., and Sonveaux, P. (2016). Monocarboxylate transporters in the brain and in cancer. Biochim. Biophys. Acta - Mol. Cell Res. 1863, 2481–2497.
5. Payen, V.L., Mina, E., Van Hée, V.F., Porporato, P.E., and Sonveaux, P. (2020). Monocarboxylate transporters in cancer. Mol. Metab. 33, 48–66.

Concomitant expression of two acetyl-CoA carboxylase genes in Saccharomyces cerevisiae

Humberto Pereira, Flávio Azevedo, Björn Johansson

CBMA–Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Portugal
Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal

Acetyl-coA carboxylase (ACC) catalyzes the ATP-dependent carboxylation of acetyl-CoA to produce malonyl-CoA. This is the rate-limiting step in fatty acid biosynthesis. *Saccharomyces cerevisiae* is one of the most used cell factories, however, it is not capable of generating malonyl-CoA in the quantities needed for the economically viable production of compounds such as biodiesel, 3-hydroxypropionic acid, and polyketides. Expression of *Yarrowia lipolytica ACC1* from a plasmid complemented the phenotype of a conditional *S. cerevisiae* strain with a tetracycline repressible *ACC1.* The heterologous ACC expression resulted in increased malonyl-CoA accumulation and lower specific growth rates. This effect was less pronounced when co-expression of the native ACC was allowed. Simultaneous expression of both the native and heterologous ACC genes unexpectedly resulted in higher growth rate and lower accumulation of malonyl-CoA, suggesting a regulatory role for the native gene. Results of *in-vivo* measurements of malonyl-CoA levels in the recombinant strain will be discussed.

Acknowledgments

This work was supported by the Fundacão para a Ciência e Tecnologia Portugal (FCT) through Project FatVal POCI-01-0145-FEDER-032506. CBMA was supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCTI.P. and by the ERDF through the COMPETE2020 - Programa Operacional Competitividade e Internacionalizacão (POCI). Humberto Pereira acknowledges FCT for the PhD scholarship, SFRH/BD/148722/2019.

Angiogenic properties of Transthyretin: *in vitro* and *in vivo* studies and impact in Alzheimer's Disease

Joana Saavedra[1,2], Tiago Gião[1,2], Marta Teixeira Pinto[1,3] and Isabel Cardoso[1,2]

[1] i3S - Institute of Research and Innovation in Health, University of Porto, Rua Alfredo Allen 208, 4200-135, Porto, Portugal;
[2] IBMC – Institute of Molecular and Cellular Biology, University of Porto, Rua Alfredo Allen 208, 4200-135, Porto, Portugal;
[3] IPATIMUP – Institute of Pathology and Molecular Immunology, University of Porto, Rua Júlio Amaral de Carvalho,45- 4200-135 Porto, Portugal.

Angiogenesis is the formation of new vessels by splitting or branching the pre-existing ones. This mechanism is crucial during embryonic development and adulthood, as it is involved in processes such as wound healing. On the other hand, aberrant angiogenesis is part of the pathogenicity of diseases as cancer or neurodegenerative disorders, such as Alzheimer's Disease (AD)[1].

In this work, we investigated the biological effects of transthyretin (TTR), an established neuroprotective protein in AD, in the vasculature. Using the *in vitro* tube formation assay we conclude that TTR leads to the formation of capillary-like structures in a dose dependent manner and regulates angiogenic molecules such as interleukin (IL)-6 and Angiopoietin (Ang)-2. Further *in vivo* investigation, using the chick chorioallantoic membrane (CAM) assay confirmed that TTR is an angiogenic molecule, and highlighted that the TTR neovessels are functional. We also performed *ex vivo* assays to elucidate the involvement of TTR in the vascular pathology using our transgenic mouse model of AD, established in different TTR genetic backgrounds. We found that 7-months old transgenic AD mice with TTR genetic reduction, AD/TTR+/-, exhibit decreased vessel length as compared to AD/TTR+/+ animals, in the hippocampus.

Our *in vivo* results show the involvement of TTR in angiogenesis, particularly as a modulator of vascular alterations occurring in AD. Since TTR levels and stability are early decreased in AD, its tetrameric stabilization can represent a therapeutic avenue for the early treatment of AD through the maintenance of the vascular structure.

References:

1. Vallon, M., Chang, J., Zhang, H., & Kuo, C. J. (2014). Developmental and pathological angiogenesis in the central nervous system. Cellular and Molecular Life Sciences, 71(18), 3489–3506.

Study of the metabolic conditions involved in the expression and trafficking of the human monocarboxylate transporter MCT1

Pinto J.[1], Barata-Antunes C.[1], Alves R.[1], De Beule P.[2], Moura C.[2], Sorkin A.[3], Paiva A.[1]

[1] Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal
[2] International Iberian Nanotechnology Laboratory, Braga, Portugal
[3] Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, USA

Cancer is a complex disease with a high level of incidence and mortality, being the second leading cause of death. It is expected in that the incidence of this disease in the population will increase, even in countries with more advanced health systems. So, efforts have been made in order to identify new-targeted therapies.

It has been reported that the monocarboxylate transporter MCT1 is highly expressed in multiple cancer types. MCT1 is a symporter that is responsible for the influx of lactate across the cell membrane in cancer cells, supplying their metabolism and their rapid proliferation and expansion. This makes it a possible potential target molecule for therapy as well as a useful prognostic factor. Based on that, this project had as a main purpose to study the metabolic conditions involved in MCT1 internalization in human cancer cell lines. We will present the results obtained after gene edited cells harboring MCT1 with GFP were treated with distinct compounds and subjected to deveral metabolic conditions.

The results obtained in this study intend to contribute towards a better understanding the molecular mechanisms involved in the MCT1 expression, and open novel therapeutic possibilities for osteosarcoma cancer.

References:

1. Fisel, Pascale, Elke Schaeffeler, and Matthias Schwab. 2018. “Clinical and Functional Relevance of the Monocarboxylate Transporter Family in Disease Pathophysiology and Drug Therapy”. Clin Transl Sci 00: 1–13.
2. Morris, Marilyn E., and Melanie A. Felmlee. 2008. “Overview of the Proton-Coupled MCT (SLC16A) Family of Transporters: Characterization, Function and Role in the Transport of the Drug of Abuse γ-Hydroxybutyric Acid”. AAPS Journal 10(2): 311–21.
3. Payen, Valéry L *et al. 2*019. “Monocarboxylate Transporters in Cancer”. Molecular Metabolism.
4. Pinheiro, Céline *et al.* 2012. “Role of Monocarboxylate Transporters in Human Cancers: State of the Art”. J Bioenerg Biomembr 44: 127–39.

Transthyretin tetrameric stabilization ameliorates the structural vascular alterations in an Alzheimer's Disease mouse model

Tiago Gião[1,2], Joana Saavedra[1,2], José Ricardo Vieira[1,2,3], Gemma Arsequell [4,] and Isabel Cardoso[1,2]

[1] i3S - Institute of Research and Innovation in Health, University of Porto, Rua Alfredo Allen 208, 4200-135, Porto, Portugal
[2] IBMC – Institute of Molecular and Cellular Biology, University of Porto, Rua Alfredo Allen 208, 4200-135, Porto, Portugal;
[3] Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
[4] Institute of Advanced Chemistry of Catalunya (I.Q.A.C.-C.S.I.C.), 08034 Barcelona, Spain.

It has been demonstrated that vascular defects, as the thickening of the basement membrane (BM) layer surrounding brain microvessels, can precede the onset of the other Alzheimer's Disease (AD) hallmarks features, making it an important therapeutic target[1,2].

Transthyretin (TTR) has been established as neuroprotective in AD but the underlying mechanisms are not fully understood[3–6]. In AD, TTR levels and stability are decreased resulting in decreased neuroprotective capacity, while TTR tetrameric stabilization by small chemical compounds (SMCs) recovers TTR levels and enhances the TTR/Aβ binding and consequent elimination of the peptide from the brain[7–11].

Here we investigated the influence of TTR and of the administration of TTR stabilizers in the BM thickness of AD mice.

We demonstrated that 7-month-old transgenic AD mice with TTR genetic reduction, AD/TTR+/, exhibited a thicker BM than animals with normal TTR levels, AD/TTR+/+. Subsequent studies in non-transgenic (NT) littermates with the same TTR genetic reduction, clarified that this effect on the BM is exerted directly by TTR, as the same thickening was found in NT/TTR+/- compared to NT/TTR+/+. Additionally, this alteration occurred prior to amyloid deposition[12]. We also showed that Aβ oligomers increased the expression of collagen IV by endothelial cells and that TTR counteracted this effect. Accordingly, we showed that TTR reduction leads to a thicker BM in AD mice than in NT animals, strengthening the idea that TTR is neuroprotective. Finally, we showed that AD/TTR+/- mice orally treated with the SMCs iododiflunisal and resveratrol displayed a significant reduction of BM thickness when compared to non-treated littermates.

Our results show the involvement of TTR as a modulator of vascular alterations occurring in AD. Since TTR is decreased early in AD, its tetrameric stabilization can represent a therapeutic avenue for the early treatment of AD through the maintenance of the vascular structure.

References:

1. Iturria-Medina, Y. *et al.* Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat. Commun.* **7**, 11934 (2016).
2. Merlini, M., Meyer, E. P., Ulmann-Schuler, A. & Nitsch, R. M. Vascular β-amyloid and early astrocyte alterations

impair cerebrovascular function and cerebral metabolism in transgenic arcAβ mice. *Acta Neuropathol.* **122**, 293–311 (2011).

3. Oliveira, S. M., Ribeiro, C. A., Cardoso, I. & Saraiva, M. J. Gender-dependent transthyretin modulation of brain amyloid-β Levels: Evidence from a mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* **27**, 429–439 (2011).
4. Costa, R., Gonçalves, A., Saraiva, M. J. & Cardoso, I. Transthyretin binding to A-Beta peptide - Impact on A-Beta fibrillogenesis and toxicity. *FEBS Lett.* **582**, 936–942 (2008).
5. Costa, R., Ferreira-da-Silva, F., Saraiva, M. J. & Cardoso, I. Transthyretin protects against A-beta peptide toxicity by proteolytic cleavage of the peptide: A mechanism sensitive to the kunitz protease inhibitor. *PLoS One* **3**, (2008).
6. Alemi, M. *et al.* Transthyretin participates in beta-amyloid transport from the brain to the liver - involvement of the low-density lipoprotein receptor-related protein 1? *Sci. Rep.* **6**, (2016).
7. Cotrina, E. Y. *et al.* Calorimetric Studies of Binary and Ternary Molecular Interactions between Transthyretin, Aβ Peptides, and Small-Molecule Chaperones toward an Alternative Strategy for Alzheimer's Disease Drug Discovery. *J. Med. Chem.* **63**, 3205–3214 (2020).
8. Ribeiro, C. A., Saraiva, M. J. & Cardoso, I. Stability of the Transthyretin Molecule as a Key Factor in the Interaction with A-Beta Peptide - Relevance in Alzheimer's Disease. *PLoS One* **7**, (2012).
9. Ribeiro, C. A. *et al.* Transthyretin stabilization by iododiflunisal promotes amyloid-β peptide clearance, decreases its deposition, and ameliorates cognitive deficits in an Alzheimer's disease mouse model. *J. Alzheimer's Dis.* **39**, 357–370 (2014).
10. Santos, S. D. *et al.* CSF transthyretin neuroprotection in a mouse model of brain ischemia. *J. Neurochem.* **115**, 1434–1444 (2010).
11. Rios, X. *et al.* Radiochemical examination of transthyretin (TTR) brain penetration assisted by iododiflunisal, a TTR tetramer stabilizer and a new candidate drug for AD. *Sci. Rep.* **9**, 1–11 (2019).
12. Santos, L. M. *et al.* Resveratrol administration increases transthyretin protein levels, ameliorating AD features: The importance of transthyretin tetrameric stability. *Mol. Med.* **22**, 597–607 (2016).

The role of cerebellar synaptic (dys)function in the neuropathology of Spinocerebellar Ataxia Type 3

Vilaça-Ferreira, AC[1,2]; Beltrão, N[3,4]; Duarte-Silva, S[1,2]; Carvalho, AL[4];
Neves-Carvalho, A[1,2,5] and Maciel, P[1,2]

[1] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710 -057 Braga, Portugal

[2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

[3] Synapse Biology Group | CNC – Center for Neuroscience and Cell Biology, University of Coimbra

[4] Doctoral Program in Experimental Biology and Biomedicine (PDBEB), Interdisciplinary Research Institute, University of Coimbra

[5] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Brain functioning relies on the unidirectional flow of information between neurons, which is orchestrated at synapses. In several neurodegenerative disorders, neuronal death is majorly observed at later stages of the disease, at specific brain regions, pointing to neuronal dysfunction as an early disease event underlying pre-symptomatic stages1. This may be a useful biomarker in clinics, enabling starting of treatment before the symptoms appear, which may halt or delay disease onset. Indeed, cumulative evidence points for synapse malfunctioning as a pathogenic event preceding neuronal degeneration in the aforementioned diseases, including polyglutamine disorders2–4. Spinocerebellar Ataxia Type 3 (SCA3) is a dominant autosomal polyglutamine disease, for each no effective treatment is available5. However, the involvement of synaptic (dys)function in SCA3, particularly at the cerebellum, a key disease-affected region in SCA3, remains unclarified. Therefore, we aim to explore the involvement of synaptic changes in SCA3 and correlate them with disease onset and progression. For this, we will perform a detailed characterization of the synapse in a SCA3 mouse model6 at pre- and symptomatic stages by analyzing the cerebellar synaptic proteome and alterations in synaptic networks through mass-spectrometry analysis, in parallel with a general evaluation of the cerebellar architecture – by assessing the cerebellar dendritic arborization by Golgi staining. A more detailed assessment of the synaptic morphology will be conducted using transmission electron microscopy imaging. Moreover, the functional status of the synapses will be evaluated in cerebellar primary cultures, by inspecting both synaptic receptors trafficking and vesicles recycling. Untangling the neuronal communication in SCA3 will contribute for our understanding of the disease pathogenic mechanisms and help to pinpoint disease-relevant biomarkers for future pre-clinical trials.

References:

1. Dugger, B. N.; Dickson, D. W. Pathology of Neurodegenerative Diseases. *Cold Spring Harb. Perspect. Biol.* **2017**, a028035. https://doi.org/10.1101/cshperspect.a028035.
2. Lieberman, A. P.; Shakkottai, V. G.; Albin, R. L. Polyglutamine Repeats in Neurodegenerative Diseases. *Annu. Rev. Pathol.* **2019**, *14*, 1–27. https://doi.org/10.1146/annurev-pathmechdis-012418-012857.
3. Bae, J. R.; Kim, S. H. Synapses in Neurodegenerative Diseases. *BMB Rep.* **2017**, *50* (5), 237–246. https://doi.org/10.5483/BMBRep.2017.50.5.038.
4. Wishart, T. M.; Parson, S. H.; Gillingwater, T. H. Synaptic Vulnerability in Neurodegenerative Disease. *J. Neuropathol. Exp. Neurol.* **2006**, *65* (8), 733–739. https://doi.org/10.1097/01.jnen.0000228202.35163.c4.

5. Da Silva, J. D.; Teixeira-Castro, A.; Maciel, P. From Pathogenesis to Novel Therapeutics for Spinocerebellar Ataxia Type 3: Evading Potholes on the Way to Translation. *Neurotherapeutics* **2019**, *16* (4), 1009–1031. https://doi.org/10.1007/s13311-019-00798-1.
6. Silva-Fernandes, A.; Duarte-Silva, S.; Neves-Carvalho, A.; Amorim, M.; Soares-Cunha, C.; Oliveira, P.; Thirstrup, K.; Teixeira-Castro, A.; Maciel, P. Chronic Treatment with 17-DMAG Improves Balance and Coordination in a New Mouse Model of Machado-Joseph Disease. *Neurother. J. Am. Soc. Exp. Neurother.* **2014**, *11* (2), 433–449. https://doi.org/10.1007/s13311-013-0255-9

Effects of Ganoderma lucidum in an animal model of obesity

Catarina Castro-Ribeiro[1], Rita Silva-Reis[1], Mariana Mendes-Gonçalves[1], O. Taofiq[3], L. Barros[3], T. Martins[1], M.J. Pires[1], M.L. Pinto[2], I.C.F.R. Ferreira[3], E. Rosa[1], Paula A. Oliveira[1]

[1]CITAB, UTAD, Portugal
[2]CECAV, UTAD, Portugal
[3]CIMO, IPB Bragança, Portugal

Obesity is considered an epidemic disorder and studies using natural compounds like those found in *Ganoderma lucidum* (GL) have been useful to understand and develop therapies to combat this disease[1]. The aim of this study was to evaluate the effect of GL in Lee index variation and on the liver in an animal model of obesity. All ethical issues were followed (approval nº 8776). Forty-eight male C57BL/6J mice were acquired and divided into 5 groups: G1-Western Diet 0.2 % Cholesterol (WD); G2-Western Control (WC); G3-WD+0.7 % g/kg GL; G4-WD+1.4 %g/kg GL; G5 WD+2.8 % g/kg of GL. At 1st,7th and 13th weeks of study, the animals were measured to calculate the Lee Index (cubicle root of the weight (g)/the nasoanal length (cm)x1000). Obesity was defined by a Lee index>310. Animals were sacrificed after 13 weeks and the liver was used for genotoxicity assessment. The chemical composition of the extract was profiled by HPLC-DAD-ESI/MS. Ganoderic acid H and *p*-hydroxibenzoic acid were the main triterpenic and phenolic acids found in the extract, respectively. Lee index values show that all animals became obese. However, at the end of the 13th week, groups supplemented with GL were the only ones where the Lee's index decreased. Genetic Damage Index (GDI) mean of G1 was significantly higher than all other experimental groups and G5 the group with the lowest GDI. Our results suggest that GL dietary supplementation can decrease animal obesity and the genetic damage caused by hyperlipidemia.

References:

1. Isabel C.F.R. Ferreira ÂF, Heleno SA. Chemical, Nutritional, and Bioactive Potential of Mushrooms. *Edible Med Mushrooms*. Published online 2017:455-501. doi:10.1002/9781119149446.ch21

Retinoic Acid signaling coordinates the metabolic component of lung branching morphogenesis

Hugo Fernandes-Silva[1,2,3], Marco G. Alves[4,5], Jorge Correia-Pinto[1,2,6], Pedro F. Oliveira[7], Catarina C. F. Homem[3], Rute S. Moura[1,2]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal
[2]ICVS/3B's - PT Government Associate Laboratory, 4710-057 Braga/Guimarães, Portugal
[3]CEDOC - Chronic Diseases Research Center, NOVA Medical School/Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, 1150-082 Lisbon, Portugal
[4]Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal
[5]Department of Microscopy, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal
[6]Department of Pediatric Surgery, Hospital of Braga, 4710-243 Braga, Portugal
[7]QOPNA & LAQV, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Retinoic Acid (RA) signaling is crucial for pulmonary development since it regulates lung patterning and early-branching morphogenesis. Recently, the metabolic profile of lung branching was described, showing a glycolytic lactate-based metabolic preference. Here, we studied the impact of RA signaling modulation on the metabolic component of lung development during the early-branching stages. We used an *ex vivo* lung explant culture system, and embryonic chicken lungs (*Gallus gallus*) were cultured under the following experimental conditions: DMSO, 1 μM RA (stimulation), or 10 μM of BMS493 (inhibition). The explant culture media was collected, and the consumption/production of extracellular metabolites evaluated by ^{1}H-NMR spectroscopy. Then, we collected lung explant tissues to determine the respiratory capacity by Seahorse analysis. We have also performed qPCR to assess the mitochondrial DNA copy number. Finally, we used *in situ* hybridization to define lactate dehydrogenase (*ldha* and *ldhb)* mRNA localization and relative expression levels. Results revealed that RA inhibition increases glucose consumption and alanine production when compared to RA stimulation. Conversely, RA stimulation promotes a sharp increase in pyruvate production, while RA inhibition triggers a decrease. At the mitochondrial level, RA inhibition leads to increased basal respiration and elevated ATP production. Furthermore, the mtDNA copy number assay revealed similar mitochondrial abundance for all three conditions. RA stimulation promotes increased expression levels of *ldha* and *ldhb* isoforms, whereas RA inhibition decreases their expression. Moreover, *ldha* and *ldhb* show a complementary spatial distribution that resembles proximal-distal markers. In conclusion, we describe an intimate interaction between signaling and metabolism during branching morphogenesis of the lung and demonstrate that RA signaling stimulation/inhibition differently impacts the metabolic component of lung development.

References:

1. Fernandes-Silva H, Vaz-Cunha P, Barbosa VB, Silva-Gonçalves C, Correia-Pinto J, Moura RS. 2017. Retinoic acid regulates avian lung branching through a molecular network. *Cell Mol Life Sci*. 74(24):4599-4619. doi:10.1007/s00018-017-2600-3.

2. Fernandes-Silva H, Alves MG, Correia-Pinto J, Oliveira PF, Moura RS. 2017. Lung branching morphogenesis, in chicken model, is accompanied by temporal metabolic changes. *Porto Biomed J.* 2(5):222-223. doi:10.1016/j.pbj.2017.07.111.
3. Fernandes-Silva H, Alves MG, Araújo-Silva H, Correia-Pinto J, Oliveira PF, Moura RS. 2019. A Warburg-like metabolic profile underlies early pulmonary branching morphogenesis. *FEBS Open Bio*. 9:141-142. doi:10.1002/2211-5463.12675.
4. Fernandes-Silva H, Araújo-Silva H, Correia-Pinto J, Moura RS. 2020. Retinoic Acid: A Key Regulator of Lung Development. *Biomolecules*. 10(1): pii: E152. doi:10.3390/biom10010152.

Acknowledgements:

This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020; and by the projects NORTE-01-0145-FEDER-000013 and NORTE-01-0145-FEDER-000023, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Hugo Fernandes-Silva was supported by a doctoral fellowship (PD/BD/137655/2018) from FCT, as part of the Inter-University Doctoral Programme in Ageing and Chronic Disease (PhDOC).

Contribution to the study of carcinogenesis of colorectal cancer using a chemically induced animal model

Rita Silva-Reis[1], Catarina Ribeiro[1], Mariana Gonçalves[1], Tiago Ferreira[1], Francisco Peixoto[2], Adelina Gama[3], Rita Ferreira[4], Paula Oliveira[1]

[1]Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB),
[2]Chemistry Centre,
[3]Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[4]Associated Laboratory for Green Chemistry (REQUIMTE), Department of Chemistry, University of Aveiro, Aveiro, Portugal

Colorectal Cancer (CRC) is one of the most common cancers worldwide, being more incident in men than in women [1]. Rodents have been used to study CRC carcinogenesis for almost 80 years [2]. Our team aimed to characterize CRC carcinogenesis in a chemically induced animal model.

Twenty-nine male Wistar rats were randomly assigned to two control groups (CTRL1 and 2), who were administrated with EDTA-saline, and two induced groups (CRC1 and 2), who were administrated with 1,2-Dimethylhydrazine (DMH) (40mg/kg). The CRC1 and CTRL1 groups, and the CRC2 and CTRL2 groups were sacrificed at weeks 11 and 17 after the start of administrations, respectively. At necropsy, colon and blood samples were collected for histopathology and the analysis of inflammation, catabolism, and oxidative stress. All ethical issues followed the guidelines of the Portuguese *Direção Geral de Alimentação e Veterinária* (approval number 010535).

There was no evidence of systemic inflammation in the induced animals and the circulating levels of CRP and IL-6 were higher in the CTRL groups. The presence of inflammatory infiltrate was observed in the colon of all animals, but inflammation was higher in the CRC groups. The serum concentrations of ghrelin and myostatin were also higher in the control groups. The induced groups were characterized by the presence of pre-neoplastic lesions in some animals. A benign neoplasm (adenoma) was observed in an animal of the CRC2 group. Although not statistically significant, the values of the antioxidant enzymes were higher in the colon of animals from CRC groups relative to controls ($p > 0.05$).

In conclusion, DMH predominantly induced pre-neoplastic lesions at the colon level and there was no evidence of systemic inflammation, which suggests the disease was at an early stage. To study CRC carcinogenesis in more advanced stages of the disease, this protocol must be improved by administering lower doses of carcinogen to younger animals and extend the experimental protocol.

References:

1. Arnold, M.; Sierra, M.S.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* **2017**, *66*, 683–691.
2. Rosenberg, D.W.; Giardina, C.; Tanaka, T. Mouse models for the study of colon carcinogenesis. *Carcinogenesis* **2009**, *30*, 183–196.

Acknowledgements:

This work is supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020 and FCT PhD grant 2020.04789.BD. The authors declare no conflict of interest.

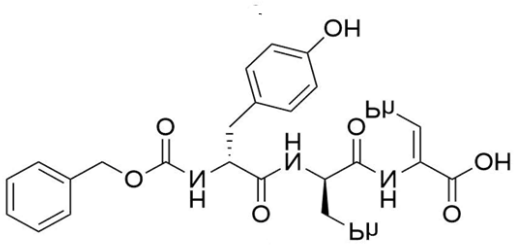
Synthesis of new peptide-based hydrogels

Carlos B.P. Oliveira[a], Peter J. Jervis[a], Sérgio R.S. Veloso[a], Paula M.T. Ferreira[a], José A. Martins[a] and Loic Hilliou[b]

[a] Centre of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal;

[b] Institute for Polymers and Composites/13N, Department of Polymer Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal.

The bottom-up approach to functional nano-objects relies on the hierarchical self-assembly of low molecular weight building blocks. Short peptides are attractive owing to easy synthesis, chemical variability, low cost and the potential to introduce biological functionality. [1-3] Susceptibility to enzymatic hydrolysis is the main limitation of these materials. Herein we report the synthesis and characterization of a tripeptide hydrogelator with a dehydrophenylalanine residue to impart proteolytic resistance and a N-benzyloxycarbonyl group (*N*-benzyloxycarbonyl-L-tyrosinyl-L-phenylalanyl-*Z*-dehydrophenylalanine; Cb*z*-L-Tyr-L-Phe-*Z*-ΔPhe-OH).



The hydrogelation conditions for this compound were established and the hydrogel was characterized using STEM, rheology and fluorescence spectroscopy. The results indicate that this hydrogel can be useful for biomedical applications.

References:

1. Kopecek, J. and Yang, J. (2008). Peptide-directed self-assembly of hydrogels. Actabiomaterilia: 805-816.
2. Jonker, A.M., Dennis, Lowik, D.W. and van Hest, C.M.(2011). Peptide- and Protein-based hydrogels. Chemistry of materials,24: 759-773.
3. Fleming, S. and Ulijn, R.V. (2014). Design of nanostructures based on aromatic peptide amphiphiles. Chemical society reviews, 43: 8150-8177.

Acknowledgments:

This work was funded by National Funds through FCT-Portuguese Foundation for Science and Technology under the Project PTDC/QUI-QOR/29015/2017 and CQ/UM UID/QUI/00686/2013 and UID/QUI/0686/2016. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

Boolaamphiphilic dehydrodipeptide hydrogels for drug delivery applications.

Carolina Amorim*, Peter Jervis, Juliana Andrade, Paula M.T. Ferreira, José A. Martins

* - Chemistry Center of University of Minho, campus de Gualtar, 4710-057 Braga.

Self-assembly of nanometric structures from molecular building blocks is an effective way to make new functional materials for biological and technological applications. In this work we synthesized new dimeric bolaamphiphilic dehydrodipeptides, containing phenylalanine connected to a dehydroamino acid residue at the C-terminus. The *N*-terminus of the dipeptide was connected to both ends of a bifunctional central aromatic moiety, namely 1,4-benzenedicarboxylic acid and 1,3-benzenedicarboxylic acid. The potential use of these new compounds as hydrogelators was evaluated. The results showed that these compounds synthesised behave as efficient molecular hydrogelators, forming hydrogels with minimum gelation concentrations of 0.3-0.8 *wt*%.

The self-assembly of these hydrogelators was investigated by STEM microscopy technique, revealing different shapes depending on the N-aromatic moiety. STEM microscopy revealed that the hydrogels are composed by fibers, ribbons and even sheres. Circular dichroism spectroscopy was also performed in order to evaluate the aggregation of the peptides into characteristic secondary structures.

Acknowledgments:

This work was funded by National Funds through FCT-Portuguese Foundation for Science and Technology under the Project PTDC/QUI-QOR/29015/2017 and CQ/UM UID/QUI/00686/2013 and UID/QUI/0686/2016. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

Magnetic dehydropeptide-based self-assembled injectable hydrogels: towards cancer theranostic agents combining hyperthermia, MRI imaging and drug-delivery capabilities

Teresa Pereira[a], Juan Gallo[b], Manuel Bañobre-López[b], Loic Hilliou[c], Paula M.T. Ferreira[a], and José A. Martins[a]

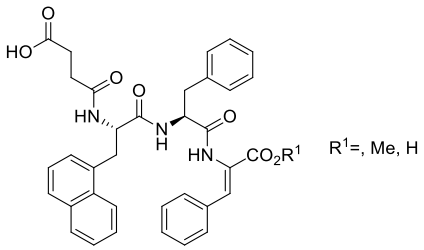
[a]Centre of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; jmartins@química.uminho.pt
[b]International Iberian Nanotechnology Laboratory (INL), Av. Mestre José Veiga s/n, 4715- 330 Braga, Portugal
[c]Institute for Polymers and Composites, Department of Polymer Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal

Magnetic Resonance Imaging (MRI) is at the forefront of clinical imaging: MRI is strictly non-invasive and depth independent and provides 3D images of soft tissues with high spatial-temporal resolution.

Magnetic Hyperthermia is emerging as a powerful cancer therapeutic modality. Magnetic induction hyperthermia is based on the exposure of magnetic nanoparticles to alternating magnetic fields. Heat generation results from magnetic hysteresis loss and Nèell and Brownian relaxation mechanisms. Tumour hyperthermia therapy is very attractive due to the benign nature of magnetic fields, allied to non-invasive and depth-independent localised heating trigged by external magnetic fields. Tumour cells are more heat-sensitive than non-tumours cells. Temperatures around 41°C are lethal to cancer cells. Superparamgnetic Iron Oxide Nanoparticles (SPION) are effective Contrast Agents for both T_{2w} MRI and Magnetic Hyperthermia.

Supramolecular (self-assembled) hydrogels based on low molecular weight peptides are the new paradigm biomaterials: porous soft biocompatible materials made of highly hydrated fibrous 3D nanostructures, reminiscent of the extracellular matrix. Our research group developed self-assembled hydrogels based on dehydrodipeptides *N*-capped with naproxen (Npx, a NSAID drug). Dehydropeptide-based hydrogels exhibit resistance to proteolysis, are biocompatible and suitable nanocarriers for delivery of incorporated drugs.[1] Recently, we demonstrated that incorporation of SPION endows dehydropeptide-based hydrogels with hyperthermia and MRI reporting properties.[2]

In this communication we report novel supramolecular hydrogels prepared by co-assembly of dehydropeptides and SPION.



Dehydropeptides

The hydrogels are characterised regarding co-assembly micro- nanostructure (STEM) and rehologial properties. The co-assembled hydrogels are characterized as Contrast Agents for MRI ($T_{1,2}$ phantoms and relaxation maps, 120 MHz, 37 °C) and as agents for magnetic hyperthermia and drug delivery.

References:

1. H. Vilaça *et al.*, *Biomacromolecules*, **2015,** 16, 3562.
2. Carvalho *et al.*, *Nanomaterials*, **2019**, 9, 541.

Acknowledgments:

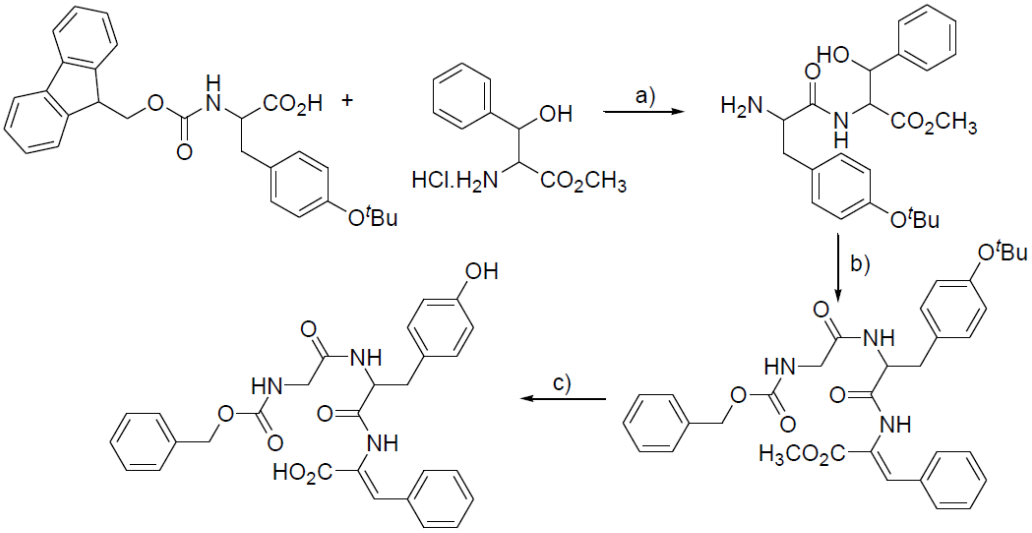
The authors acknowledge the FCT under the Project PTDC/QUI-QOR/29015/2017 and CQ/UM UID/QUI/00686/2013 and UID/QUI/0686/2016 projects.

Hydrogels based on tyrosine dihydropeptides

Silva, A.V.G, Ferreira. P.M., Martins, J.A.

Chemistry Center, University of Minho, Gualtar Campus, 4710-057 Braga, Portugal

Small molecules with the appropriate balance of hydrophobicity and hydrophylicity can selfassemble into nanofibers. The design and discovery of peptides that self-assemble to form hydrogels due to their biomedical applications have attracted considerable research attention.[1] In this work we exploit the use of small dehydropeptides with tyrosine residues as hydrogelators. The dehydropeptides with a dehydrophenylalanine were prepared from the corresponding peptides having a phenylserine residue (Scheme 1). This strategy involves a dehydration step with tert-butyldicarbonate (Boc2O) and 4-dimethylaminopyridine (DMAP) followed by treatment with N,N,N',N'-tetramethylguanidine (TMG).



Scheme 1. Synthesis of a tyrosine based dehydrotripeptide hydrogelator. a) i. HBTU / HOBt, ii. Piperidine in DMF; b) i. Z-Gly-OH, DCC / HOBt, ii. Boc_2O / DMAP, TMG; c) i. TFA, ii) NaOH 1M.

The self-assemble capability of the dehydropeptide hydrogelators was evaluated together with the *in vitro* stability of the hydrogels. These nanostrutured materials were tested for biocompatibility and bioactivity and evaluated as drug delivery systems for medical textiles.

Acknowledgments:

This work was funded by National Funds through FCT-Portuguese Foundation for Science and Technology under the Project PTDC/QUI-QOR/29015/2017 and CQ/UM UID/QUI/00686/2013 and UID/QUI/0686/2016.

Optimization of culture medium composition using agro-industrial by- products for the production of Silk-Elastin-Like Proteins by Escherichia coli

Araújo L.[1,2], da Costa A.[1,2], Machado R.[1,2]

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

In recent years, research and development of biopolymers have been gaining significant momentum, driven by the need to find more innovative and promising materials with enhanced properties and less ecological impact. Recombinant protein-based polymers (rPBPs) are an example of such innovation-driven outcomes and, among them, Silk-Elastin-Like Proteins (SELPs) demonstrate unique properties and have been used for an assortment of different applications.

SELPs are composed of silk-like and elastin-like blocks and have been successfully produced and characterized in a typical biological production process at the laboratory level. But, in order to scale up at the industrial level and achieve economically feasible levels, it is important to improve efficiency by reducing the costs of protein production, but also improving overall production.

In this sense, this work targets the optimization of SELP bioproduction in batch fermentation conditions, using alternative culture media formulated with agro-industrial by-products or residues. Optimization studies were conducted using classical medium optimization techniques namely, one-factor- at-a-time (OFAT) and statistical design with central composite design (CCD). Moreover, the employment of Proton Suicide Method (PSM) was envisaged to obtain a mutant *Escherichia coli* with low organic acids (OA) production capacity, providing less stress and driving bacterial metabolism towards SELP production.

Preliminaries results demonstrate that culture media made from agro-industrial by-products or residues is highly promising, resulting in results similar to commercial media formulations. Also, a new strain of *E. coli*, obtained by PSM demonstrated greater SELP expression levels than the original strain.

Keywords:
Biopolymers; recombinant protein-based polymers; Silk-Elastin-Like protein; production optimization; Proton Suicide; Central Composite Design.

References:

Collins, T., Silva, J. A., Costa, A. da, Branca, F., Machado, R., & Casal, M. (2013). Batch production of a silk-elastin-like protein in *E. coli BL21(DE3)*: key parameters for optimisation. Microbial Cell Factories, 12 (1), 21.

Machado, R., Azevedo-Silva, J., Correia, C., Collins, T., Arias, F. J., Rodrguez-Cabello, J. C., & Casal, M. (2013). High level expression and facile purification of recombinant silk-elastin-like polymers in auto induction shake flask cultures. Amb Express, 3 (1), 11.

Acknowledgments:

This work was supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) and the project FunBioPlas (ERA-IB-2-6/0004/2014) funded by national funds through FCT I.P. (Fundação para a Ciência e Tecnologia, Portugal) and by ERDF (European Regional Development Fund) via COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI, Portugal