Chem&BioChem

Postgraduate Students Meeting

Ciências ULisboa

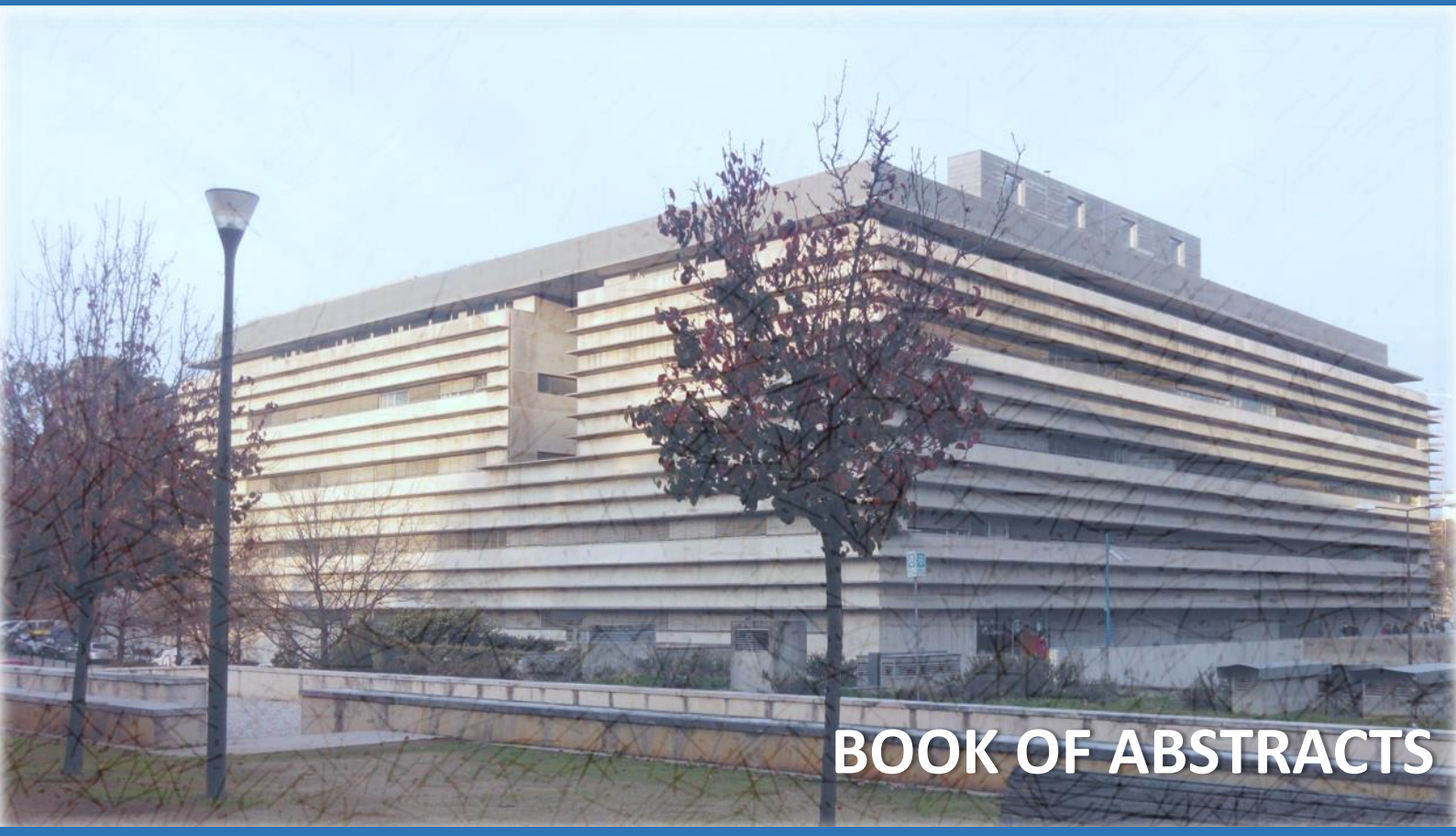
Faculdade de Ciências da Universidade de Lisboa

2017

FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA

Lisbon, PORTUGAL

February 9th 2017



BOOK OF ABSTRACTS

Edited by: Organizing Committee of the Chem&BioChem Postgraduate Students Meeting 2017

BOOK OF ABSTRACTS

Chem&BioChem Postgraduate Students Meeting 2017

February 9th 2017

Faculdade de Ciências da Universidade de Lisboa

Lisbon, Portugal

Content

Organizing committee / Advisory board / Staff	1
Sponsors	4
Scientific program	5
Plenary Abstracts	6
Chemistry Oral Communication Abstracts	9
Biochemistry Oral Communication Abstracts	13
Biochemistry Flash Communication Abstracts	17
Chemistry Flash Communication Abstracts	23
Poster Communication Abstracts	29

Organizing committee / Advisory board / Staff

Members of the Organizing Committee

Ana Marta de Matos, PhD student in Medicinal Chemistry at FCUL

Beatriz Barrocas, PhD student in Chemistry – Nanomaterials at FCUL

Filipa Santos, PhD student in Biochemistry – Molecular Biophysics at FCUL

Hugo Santos, BioSYS PhD student in Systems Biology at FCUL

Leonor Côrte-Real, PhD student in Chemistry – Inorganic Chemistry at FCUL

Samir Ahmad, PhD student in Analytical Chemistry at FCUL

Members of the Advisory Board

MSc Coordinators in Chemistry

Fernando Santos | Assistant Professor

Estrela Melo Jorge | Assistant Professor

Maria Luísa Moita | Assistant Professor

MSc Coordinators in Technological Chemistry

Maria Helena Garcia | Associate Professor with Habilitation

Ana Paula Carvalho | Assistant Professor

Maria José Lourenço | Assistant Professor

MSc Coordinators in Biochemistry

Francisco Pinto | Assistant Professor

PhD Coordinators in Chemistry

Ana Paula Paiva | Assistant Professor

Ana Silveira Viana | FCT Investigator

PhD Coordinators in Biochemistry

Maria Luísa Serralheiro | Assistant Professor with Habilitation

Other members of the Advisory Board (BioSYS)

Carlos Farinha | Assistant Professor with Habilitation

Margarida Gama-Carvalho | Assistant Professor

Other members of the Advisory Board (Catsus)

Carla D. Nunes | Principal Investigator, FCT

Staff

Beatriz Duarte | 3rd year in Biochemistry

Diogo Ferreira | 3rd year in Chemistry

Gonçalo Trindade | 2nd year in Biochemistry

Patrícia Calado | 3rd year in Chemistry

Rita Pisco (Official photographer) | 3rd year in Biochemistry

Tânia Rodrigues | 3rd year in Biochemistry

Sponsors

We would like to thank the sponsors for their generous support.

Associação de Trabalhadores da Faculdade de Ciências

CGD, Caixa Geral de Depósitos

FCUL, Faculdade de Ciências da Universidade de Lisboa

Hovione

LaborSpirit

Paralab

Pastéis de Belém

Sapec Agro

Solítica

Soquímica

SPQ, Sociedade Portuguesa de Química

Sumol+Compal

UL, Universidade de Lisboa

Scientific program

MORNING SESSION

8.30 — 9.00 | Registration

9.00—9.15 | Opening Session

José Artur Martinho Simões | Director of FCUL
Ana Maria Ponces-Freire | President of Departamento de Química e Bioquímica— FCUL

9.15 — 10.05 | Plenary Lecture

Chair: Ana Maria Ponces-Freire
Alexandre Quintas | Laboratório de Ciências Forenses e Psicológicas Egas Moniz | *A chemical and biochemical approach towards the understanding of the toxicological impact of synthetic cannabinoids consumption*

10.05 — 10.35| Coffee Break and Poster Session

10.35 — 11.35 | Oral Communications (CHEMISTRY)

Chair: Helena Garcia
10.35 OC1 Ana Marta de Matos | *Tuning C-Glucosyl Flavonoid Analogues into CNS-Targeted Molecules Against $A\beta_{1-42}$-Induced Neurodegeneration*
10.55 OC2 Leonor Côrte-Real | *New Self-Assembled Polymer-Methyl Compounds: Highly Efficient Anticancer Vesicles*
11.15 OC3 Alessandra Ide | *Novel Bar Adsorptive Microextraction Devices for the Analysis of Antidepressive Agents in Water Matrices*

11.35 — 12.05 | Poster Session

12.05 — 12.30| Flash Communications (BIOCHEMISTRY)

Chair: António Ferreira
12.05 FC1 António Flor | *Interaction of Plant Polyphenols with Lipid Bilayers: a Fluorescence Spectroscopy Characterization*
12.10 FC2 Cláudio Piedade | *How to Disassemble a Virus Capsid: a Computational Approach*
12.15 FC3 Hugo Santos | *Untangling the Gene Networks for Motor Neuron Degeneration: from Disease Model Transcriptomes to Cellular Systems*
12.20 FC4 Joana Cristóvão | *The Neuronal S100B Protein as a Novel Modulator of Amyloid-β Aggregation in Alzheimer's Disease*
12.25 FC5 Margarida Quaresma | *CFTR in the Maintenance of Epithelial Differentiation in the Airways*

AFTERNOON SESSION

13.45 — 14.35 | Plenary Lecture

Chair: Rodrigo de Almeida
Radoslaw Starosta | University of Wroclaw| *A story about aminomethylphosphanes. Gaining experience throughout collaborations*

14.35 — 16.05 | Round Table

Job Opportunities for Postgraduates: Academia vs. Industry

Filipe Duarte | HOVIONE
Miguel Prudêncio | IMM | RoPlaVac
Tino Rossi | BIAL
Maria José Calhorda | FCUL
Carlos Castro | FCUL

Moderated by Jorge Correia and Margarida Amaral

16.05 — 16.35| Coffee Break and Poster Session

16.35 — 17.35 | Oral Communications (BIOCHEMISTRY)

Chair: Margarida Gama Carvalho
16.35 OC4 André Bastos | *Insights into the Involvment of Feeding Cycle in the Physiology of Hippocampal Na^+ Channels*
16.55 OC5 Daniel Olivença | *A Mathmatical Model of the Phosphoinositide Pathway in Human Pulmonary Epithelial Cells*
17.15 OC6 Sara Canato | *Modulation of Protein Traffick Networks to Rescue F508DEL-CFTR from Endoplasmic Reticulum*

17.35 — 18.00| Flash Communications (CHEMISTRY)

Chair: Filomena Martins
17.35 FC6 Bárbara Anes | *Chemical and Metrological Challenges to the Quality of Seawater*
17.40 FC7 Luís Almeida | *Immobilization of Laccase and Magnetite Nanoparticles on a Biocompatible Polymer for Electrochemical Biosensing*
17.45 FC8 Olga Ferreira | *Non-Releasing Antifoulding Coatings: an Eco-Friendly Strategy for Surfaces Protection against Biofoulding*
17.50 FC9 Rodrigo Osawa | *Identification of Citalopram Transformation Products by LC QTOF-MS*
17.55 FC10 Sara Realista | *A Capture of Small Molecules by Metal-Organic Structures*

18.00 — 18.10 | Closing Session & Awards

Plenary Abstracts

PL1 - A chemical and biochemical approach towards the understanding of the toxicological impact of synthetic cannabinoids consumption

Alexandre Quintas[1,2]

(1) Laboratório de Ciências Forenses e Psicológicas Egas Moniz, Campus Universitário, Qta. Da Granja, 2829-511 Monte da Caparica , Portugal*;* ***(2)*** Centro de investigação interdisciplinar Egas Moniz, Campus Universitário, Qta. Da Granja, 2829-511 Monte da Caparica, Portugal*;*

lcforenses@egasmoniz.edu.pt

The use of novel psychoactive substances (NPS) has been increasing and has become a major public health concern. The acronym NPS stands for designed substances such as cathinones or synthetic cannabinoids. "K2" and "Spice" have been found to contain synthetic cannabinoids[1] a class of NPS that have emerged as popular alternatives to marijuana. These substances are easily available on the Internet as harmless products commercially classed as herbal blends or Chemical Research Products. In spite of the presence of a label on the package stating "not for human consumption", there are a significant number of deaths related with the intake of synthetic cannabinoids[2]. However the striking feature about synthetic cannabinoids has been the way in which this synthetic cannabinoids has evolved and adapted since their first identification in the European market in 2008. In fact this has lead the EMCDA to mention in its last report that “It is clear that the innovative chemical substitution patterns that have characterized this phenomenon mean that continued close monitoring of new developments in the field”[3]. However the static knowledge obtain by observing it is not enough. To become one step ahead from clandestine laboratories it is compulsory to understand the chemical evolution patterns of synthetic cannabinoids. Moreover, understand the toxicological impact of this NPS in a short time window, as they are intake by users, is paramount to provide tools for the authorities to schedule such substances[4]. This is the present mission of the Toxicological Department at the Egas Moniz Forensic and Psychological Sciences Laboratory.

Acknowledgments: Professor José Martins dos Santos† for the unconditional support to this project

References

[1] Auwärter, V., Dresen, S., Weinmann, W., Müller, M., Pütz, M., and Ferreirós, N. (2009) “Spice” and other herbal blends: harmless incense or cannabinoid designer drugs? *J. Mass Spectrom. 44*, 832–837.

[2] Brenneman, R., Papsun, D. M., Logan, B. K. and Neavyn, M. J. (2016), ‘Death-like slumber: Toxic outbreak of AB-FUBINACA’, Journal of Medical Toxicology, 12(1), p. 39.

[3] PERSPECTIVES ON DRUGS I Synthetic cannabinoids in Europe (2016) European Monitoring Centre for Drugs and Drugs Addiction.

[4] Couceiro, J., Bandarra, S., Sultan, H., Bell, S., Constantino, S., Quintas, A. (2016) Toxicological impact of JWH-018 and its phase I metabolite N-(3-hydroxypentyl) on human cell lines, Forensic Sci Int. 2016 264:100-5.

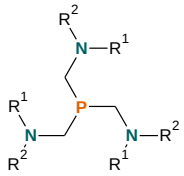
PL2 - A story about aminomethylphosphanes.

Gaining experience throughout collaborations.

Radosław Starosta [1]

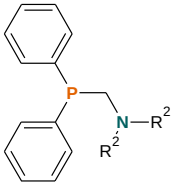
(1) *Faculty of Chemistry, University of Wrocław, Poland*

radoslaw.starosta@chem.uni.wroc.pl



The synthesis of tris(aminomethyl)phosphines from tris(hydroxymethyl)phosphine and dialkylamines was first described by Coates and Hoye in 1960. A wide range of possible aminomethylphosphines and their structural diversity can lead to multiple applications in chemistry, biochemistry and biomedical sciences. However, despite a big number of synthesized aminomethylphosphines with aromatic substituents and a few known examples of the highly water-soluble ligands prepared from aliphatic amines or aminoacids, the coordination chemistry of this class of ligands remained rather unexplored.

This encouraged us to undertake studies on the properties of this class of compounds and their complexes with platinum(II), ruthenium(II) and copper(I) ions. Using a variety of different physico-chemical and biological techniques we proved that Cu(I) complexes of a general formula [Cu{(pseudo)halogen}NCS)(diimine)(phosphine)] are very interesting as templates for biologically active compounds. Their properties are most probably a function of their mixed molecular structure: a coordinated diimine molecule capable of π-stacking interactions with rings of tryptophan, tyrosine and phenylalanine and intercalation to DNA or RNA chain and a phosphine ligand with an easily modifiable steric properties and hydrophilicity.



Changing slightly our approach, in the next step we started to study the simpler ligands with only one aminomethyl substituent, i.e. diphenylphoshinomethyl derivatives of amines or peptides of documented biological activity. Copper(I) complexes with diimines and derivatives of selected fluoroquinolone antibiotics (i.e. ciprofloxacin, norfloxacin, lomefloxacin and sparfloxacin) proved to be interesting as potential anticancer agents.

Currently, we are starting to working on new derivatives of ketoconazole and other azoles as antifungal agents targeting sphingolipids in the plasma membrane. This bilateral project Poland-Portugal is the perfect opportunity to expand the studies on fungal sphingolipids to the action of azole compounds, with the added-value of being new compounds.

Acknowledgments: *Author would like to thank to all the co-authors and co-workers from Department of Veterinary Microbiology and Department of Biotechnology and Food Microbiology - Wroclaw University of Environmental and Life Sciences (Wroclaw, Poland), Department of Medical Biochemistry - Wroclaw Medical University (Wrocław, Poland), Department of Microbiology, Institute Genetics and Microbiology - University of Wroclaw (Wrocław, Poland); Anorganische Chemie I-Festkörperchemie und Materialien - Ruhr-Universität Bochum (Bochum, Germany), Faculty of Chemistry, Jagiellonian University (Kraków, Poland) and Molecular Biophysics Group - Faculdade de Ciências da Universidade de Lisboa (Lisbon, Portugal). Also, I would like to acknowledge the hosting and support from Departamento de Química e Bioquímica and Centro de Química e Bioquímica, Universidade de Lisboa.*

Chemistry
Oral Communication
Abstracts

OC1 - TUNING *C*-GLUCOSYL FLAVONOID ANALOGUES INTO NEW CNS-TARGETED MOLECULES AGAINST $A\beta_{1-42}$-INDUCED NEURODEGENERATION

Ana M. Matos,[1,2] Joana S. Cristóvão,[3] Ana S. Viana,[1] Teresa Man,[4] Cláudio Gomes,[3] David Evans,[4] Magnus Walter[4] and Amélia P. Rauter[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa;* ***(2)*** *CEDOC Chronic Diseases, Nova Medical School;* ***(3)*** *Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa;* ***(4)*** *Department of Chemistry,Erl Wood Manor, Eli Lilly UK.*

amamatos@fc.ul.pt

Flavonoids have been extensively studied for a variety of medical applications, and many of them were shown to display neuroprotective effects associated with multitarget mechanisms of action in the context of Alzheimer's disease (AD) [1-4]. In this study, we were interested in modifying natural flavonoids in order to convert them into drug-like and CNS-targeted molecules with potential against AD, namely through *C*-glycosylation reactions and modifications in ring B.

We firstly investigated a number of naturally occurring catechol-type and non-catechol-type flavonoids, together with a synthetic chromone analogue, aiming at disclosing structural requirements for their activity as protein-protein interaction inhibitors (PPII) against amyloid β 1-42 ($A\beta_{1-42}$), a key partaker in the pathophysiology of AD. Using thioflavin T (ThT) monitored kinetics and atomic force microscopy (AFM), we selected the flavone skeleton as our preliminary lead scaffold for its greater potential to reduce the total amyloid fibril mass and, very importantly, the formation of small oligomers, which have been regarded as the most neurotoxic amyloid species [5].

In the light of these results, we also present the rational design and synthesis of new *C*-glucosyl flavone analogues and respective aglycones, selected on the basis of the computational study of more than 90 possible molecules, assembled in a database collection. This small library of analogues is currently being evaluated with the ultimate goal of finding new blood brain barrier (BBB)-permeable molecules that combine key features of the flavone scaffold with the known benefits of the sugar moiety [6].

Acknowledgments: *The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°612347. Fundação para a Ciência e a Tecnologia is also acknowledge for the support of the projects MULTI/UID 0612/2013, PTDC/BIM-MET/2115/2014, and for the Ph.D. grants SFRH/BD/93170/2013 (to AMM) and SFRH/BD/101171/2014 (to JSC).*

References

[1] A.M. Sabogal-Guáqueta, J.I. Muñoz-Manco, J.R. Ramírez-Pineda, M. Lamprea-Rodriguez, E. Osorio, G.P. Cardona-Gómez. *Neuropharmacology*, **2015**, 93, 134.

[2] L. Zhao, J.L. Wang, R. Liu, X.X. Li, L. Zhang. *Molecule*s, **2013**, 18(8), 9949.

[3] J.S. Choi, M.N. Islam, M.Y. Ali, Y.M. Kim, H.J. Park, H.S. Sohn, H.A. Jung. *Arch. Pharm. Res.*, **2014**, 37(10), 1354.

[4] M.A. Ansari, H.M. Abdul, G. Joshi, W.O. Opii, D.A. Butterfield. J. Nutr. Biochem., 2009, 20(4): 269.

[5] U. Sengupta, A.N. Nilson, R. Kayed. *EBioMedicine*, **2016**, 6, 42.

[6] A.M. Matos, C. Dias, A.P. Rauter in *Coupling and Decoupling of Diverse Molecular Units in Glycosciences* (Eds. Z. Witczak and R. Bielski) Springer Verlag, Germany, 2016 (in press).

OC2 - NEW SELF-ASSEMBLED POLYMER-METAL COMPOUNDS: HIGHLY EFFICIENT ANTICANCER VESICLES

Leonor Côrte-Real,[1] Raquel de la Campa,[2] Alejandro P. Soto,[2] Ana S. Viana,[3] Catarina Roma Rodrigues,[4] Alexandra R. Fernandes,[4] M. Helena Garcia[1] and Andreia Valente[1]

(1) *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Facultad de Química, Universidad de Oviedo, Julián Clavería s/n, 33006 Oviedo, Spain;* ***(3)*** *Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(4)*** *UCIBIO, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal.*

leonorcreal@gmail.com

One of the major challenges in chemotherapy involves the delivery of drugs selectively to the cancer cells while sparing healthy cells and tissues. Thus, the finding of less toxic, more selective and efficient drugs to treat cancer is urgent. One approach to circumvent this problem is the design of molecules (conjugates) bearing a recognized cytotoxic drug and a biological moiety acting as a vector unit to target the cancer cell. Our research group is focused on the development of ruthenium(II) and iron(II) compounds with 'MCp'(Cp = η^5-C_5H_5) core that exhibit exceptional anticancer *in vitro* activity in several cancer cell lines.[1-4] These encouraging results, led us to explore the synthesis and biological evaluation of novel polymer-metal based compounds (PMC) with several pendant groups as potential targeting anticancer agents. These PMC provide potential tools to surmount many of the current limitations in conventional chemotherapy, including undesirable biodistribution, cancer cell drug resistance and severe side effects. Furthermore, macromolecular drugs also show improved tumor-selective targeting. Two novel PMC containing an amphiphilic copolymer with hydrophilic (polyethylene glycol – PEG) and hydrophobic (polylactide – PLA) blocks, that self-assemble into micelles when dissolved in water, encapsulating the cytotoxic 'MCp' fragment inside the micelle, have been synthesized **(Figure 1** – Proposed structure of novel PMC vesicles). These new PMC were characterized by the usual spectroscopic techniques and by Dynamic Light Scattering and the micelles were visualized by Atomic Force Microscopy, and Transmission Electron Microscopy. Preliminary results on the antiproliferative effect have showed a remarkable activity towards tested cell lines A2780 and MCF7 with IC_{50} values in the nanomolar range.

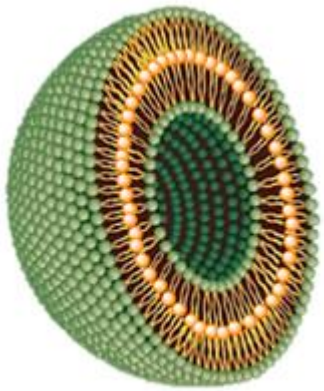


Figure 1

Acknowledgments: *This work was financed by national funds through FCT, The Portuguese Foundation for Science and Technology, within the scope of the projects IF/01302/2013, UID/QUI/00100/2013 and UID/Multi/04378/2013 and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728). Leonor Côrte-Real thanks FCT for her Ph.D. Grant (SFRH/BD/100515/2014). The authors gratefully acknowledge the COST action CM1302 (SIPs).*

References

[1] A. Valente, M.H. Garcia; F. Marques, Y. Miao, C. Rousseau, P. Zinck, Journal of Inorganic Biochemistry 2013, 127, 79-81; [2] V. Moreno, M. Font-Bardia, T. Calvet, J. Lorenzo, F. X. Alvilés, M. H. Garcia, T. S. Morais, A. Valente, M. P. Robalo, Journal of Inorganic Biochemistry 2011, 105, 241-249; [3] A. Valente, A.M. Santos, L. Côrte-Real, M.P. Robalo, V. Moreno, M. Font-Bardia, T. Calvet, J. Lorenzo, M.H. Garcia, Journal of Organometallic Chemistry 2014, 756, 52-60; [4] M.H. Garcia, A. Valente, T.S.F. Morais, A.I. Tomaz, Macromolecular Transition Metal Complexes For Treatment Of Cancer And Process For Their Preparation, PCT/IB2015/002312.

OC3 - NOVEL BAR ADSORPTIVE MICROEXTRACTION DEVICES FOR THE ANALYSIS OF ANTIDEPRESSIVE AGENTS IN WATER MATRICES

A.H. Ide[1] and J.M.F. Nogueira[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

ahide@fc.ul.pt

Bar adsorptive microextraction (BAμE) is a novel sample enrichment technique used in analytical chemistry [1], in which a bar-shaped device is used, simultaneously with a conventional Teflon magnetic stirring bar at the bottom of the sampling flask. The centripetal force promoted by the magnetic bar put the analytical device under free-floating motion just below the centre of the vortex formed ("floating sampling technology"; figure 1). During this static process, the analytes migrate by diffusion from the sample bulk and then are retained in a convenient sorbent phase, where the microextraction takes place.

BAμE is very cost-effective and easy to operate, although the back-extraction stage, performed through liquid desorption (LD), requires several manipulating steps. In the present contribution, a novel BAμE device is proposed, smaller and more flexible in comparison to the original ones, enabling a more user-friendly back extraction stage (only-single LD step) and compatible with routine analysis by using the currently autosamplers of the chromatographic systems. To assess the proposed improvements, four antidepressive agents (bupropion, trazodone, citalopram and amitriptyline) were used as model compounds.

Figure 1. BAμE operating under the floating sampling technology.

Acknowledgments: *The authors wish to thank "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (Brazil) for the PhD grant (CAPES BEX 0394-14-9) and "Fundação para a Ciência e a Tecnologia" (Portugal) for funding (UID/MULTI/00612/2013).*

References

[1] N.R. Neng, a. R.M. Silva, J.M.F. Nogueira, *J. Chromatogr. A.* **2010**, 1217, 7303-7310.

Biochemistry
Oral Communication
Abstracts

OC4 - INSIGHTS INTO THE INVOLVEMENT OF FEEDING CYCLE IN THE PHYSIOLOGY OF HIPPOCAMPAL Na^+ CHANNELS

André E.P. Bastos,[1] Rodrigo F.M. de Almeida[2] and Pedro A. Lima[3]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa Departamento de Fisiologia, Nova Medical School, Faculdade de Ciências Médicas, Lisboa;* ***(2)*** *Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa;* ***(3)*** *Departamento de Fisiologia, Nova Medical School, Faculdade de Ciências Médicas, Lisboa.*

fc32330@alunos.fc.ul.pt

Emerging information has been strengthening the hypothesis that variations of metabolism may condition brain activity. We discovered that feeding cycle influences excitability in rat hippocampal CA1 neurons [1]. In view of the functional implications of the feeding cycle, it is important to investigate further the mechanisms underlying the adaptation of intrinsic neuronal membrane properties to such metabolic conditions.

Here, electrophysiological and molecular biology methods were applied to address the involvement of voltage-gated sodium (Na^+) channels (Na_v) in the surface of acutely isolated rat hippocampal CA1 neurons. Animals were subjected to overnight fasting (*fasted*) or, subsequently, fed for 45min (*fed*). The results indicate a conspicuous effect of the physiological feeding cycle over the activation of Na^+ channels, as we detected higher current amplitude in fed neurons, supported by an increase of the conductance of Na^+ ions and an augment in the membrane expression of the isoform $Na_{v1.2}$ in hippocampal neurons of fed animals. The inactivation process is also affected, pointing to a conformational rearrangement of Na^+ channels after feeding, which might be due to alterations in the lipid composition and/or order of the plasma membrane, mainly in specialized lipid microdomains [2].

Overall, the results indicate an influence of feeding cycle in the expression and biophysics of voltage gated Na^+ channels present in rat hippocampal CA1 neurons, phenomena that account for a change in neuroexcitability.

Acknowledgments: *F.C.T. - SFRH/BD/88199/2012 (PhD project) and IF2012. Sea4Us, Biotechnology and Marine Resources company, for funding.*

References

[1] P. A. Lima, P. C. Costa, M. Mondragão, F. M. Alves, G. Costa, D. Hardy, A. Jalil and C. Auger., "Metabolic states induced by feeding/fasting influence insulin-induced excitability and levels of insulin receptor in hippocampal but not cerebellar neurons.," *8 th FENS Forum Neurosci.*, 2012.

[2] B. A. Tsui-Pierchala, M. Encinas, J. Milbrandt, and E. M. Johnson, "Lipid rafts in neuronal signaling and function.," *Trends Neurosci.*, vol. 25, no. 8, pp. 412–7, Aug. 2002.

OC5 - A MATHEMATICAL MODEL OF THE PHOSPHOINOSITIDE PATHWAY IN HUMAN PULMONARY EPITHELIAL CELLS

Daniel Vigário Olivença[1], Francisco Pinto[1] and Eberhard Voit[2]

(1) *BIOISI: Biosystems and Integrative Sciences Institute. Faculdade de Ciências da Universidade de Lisboa Campo Grande 1749-016 Lisboa, Portugal;* ***(2)*** *The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 950 Atlantic Drive, Atlanta, Georgia, 30332-2000, USA.*

dolivenca@fc.ul.pt

Phosphoinositide's are important signaling lipids in the cell membranes. We propose a computational model that accounts for all known species of phosphoinositide's in the plasma membrane of mammalian epithelial cells. It focuses, in particular, on the control of ion channels and the role of the epithelial sodium channel ENaC, which is critically involved in diseases like cystic fibrosis. The model faithfully replicates the steady state of the phosphoinositide pathway system, as well as several dynamic phenomena that had been observed and documented in the literature. Furthermore, local and global sensitivity analysis demonstrates that the model is robust to moderate alterations in any of the parameters. The model was validated against data from siRNA screens and allow us to test various novel hypotheses. With respect to ENaC, which is regulated by PI(4,5)P2, the model suggests a control strategy where the activity of the enzyme PIP5KI is decreased. This reduction in activity is most efficacious in affecting PI(4,5)P2 levels and, consequently, the functionality of ENaC.

References

[1] T. Balla, “Phosphoinositides: tiny lipids with giant impact on cell regulation,” *Physiol. Rev.*, vol. 93, no. 3, pp. 1019–1137, **2013.**

[2] J. Almaça, D. Faria, M. Sousa, I. Uliyakina, C. Conrad, L. Sirianant, L. A. Clarke, J. P. Martins, M. Santos, J. K. Heriché, W. Huber, R. Schreiber, R. Pepperkok, K. Kunzelmann, and M. D. Amaral, “High-content siRNA screen reveals global ENaC regulators and potential cystic fibrosis therapy targets,” *Cell*, vol. 154, no. 6, **2013**.

[3] T. Sasaki, S. Takasuga, J. Sasaki, S. Kofuji, S. Eguchi, M. Yamazaki, and A. Suzuki, “Mammalian phosphoinositide kinases and phosphatases,” *Prog. Lipid Res.*, vol. 48, no. 6, pp. 307–343, **2009**.

[4] E. O. Voit, *Computational Analysis of Biochemical Systems: A Practical Guide for Biochemists and Molecular Biologists*. Cambridge University Press, **2000**.

[5] G. Di Paolo and P. De Camilli, “Phosphoinositides in cell regulation and membrane dynamics.,” *Nature*, **2006**.

OC6 - MODULATION OF PROTEIN TRAFFIC NETWORKS TO RESCUE F508DEL-CFTR FROM THE ENDOPLASMIC RETICULUM

Sara Canato,[1] Ana Sofia Carvalho,[2] Hugo M. Botelho,[1] Kerman Aloria,[3] Rune Matthiesen,[2] Margarida D. Amaral,[1] André Falcão[1,4] and Carlos M. Farinha[1]

(1) *Functional Genomics and Proteostasis Group, BioISI-Biosystems and Integrative Sciences Institute, University of Lisboa, Faculty of Sciences Lisboa, Portugal* ***(2)*** *Computational and Experimental Biology Group, CEDOC - Chronic Diseases Research Center, Nova Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal* ***(3)*** *Faculdad de Cincia y Tecnologia , Universidad del Pais Vasco UPV/EHU, Vizcaya Spain* ***(4)*** *Department of Informatics, University of Lisboa, Faculty of Sciences, Lisboa, Portugal.*

sara.canato@gmail.com

Background: F508del, the most common cystic fibrosis-causing mutation (~85% of CF patients), leads to CFTR misfolding which is recognized by the endoplasmic reticulum (ER) quality control (ERQC) resulting in ER retention and early degradation [1]. CFTR traffic from the ER is mediated by specific sorting motifs that include the 4 retention motifs AFTs (arginine-framed –RXR- tripeptides) [2].

Aim: Here, we aim to identify traffic factors that regulate CFTR exit from the ER at these specific QC checkpoints.

Methods: We performed pull-down assay by co-immunoprecipitation of F508del-CFTR (with and without mutated AFT motifs) to identify and isolate the factors that interact specifically with each of these variants. The respective protein profiles were analysed by LC-MS/MS and proteins showing differential interactions were selected.

Results and Discussion: A high number of interacting proteins (~800) was identified. In those with stronger interaction with F508del-CFTR, there is an enrichment in proteins involved in RNA processing and complex organization and a decrease in those related to epithelial integrity when compared to the interactome of F508del-CFTR with abrogated AFT motifs. A subset of 52 proteins was identified as potentially involved in F508del-CFTR rescue. Several of these interactors are involved in protein trafficking and processing as well as in cell integrity and homeostasis and not previously directly associated with CFTR regulation, being currently under validation.

The identification of the specific CFTR interactors/regulators, and its validation which is in progress, will likely identify novel therapeutic targets that could be ultimately used to the benefit of CF patients.

Acknowledgements: *Work supported by UID/MULTI/04046/2013 centre grant from FCT, Portugal (to BioISI). SC is recipient of PhD fellowship SFRH/BD/5249/2014 from BioSys PhD programme from FCT – Portugal.*

References

[1] Amaral MD (2005) Pediatr Pulmonol. **39**, 479-91.

[2] Roxo-Rosa M et al (2006) *Proc Natl Acad Sci USA*, **103**, 47:17891-6.

Biochemistry
Flash Communication
Abstracts

FC01 - INTERACTION OF PLANT POLYPHENOLS WITH LIPID BILAYERS: A FLUORESCENCE SPECTROSCOPY CHARACTERIZATION

A.G. Flor,[1] Hugo A. L. Filipe,[1] Carla Sousa[1] and Rodrigo F. M. de Almeida[1]

(1) *CQB, DQB, FCUL, Campo Grande, 1749-016 Lisboa, Portugal.*

fc44207@alunos.fc.ul.pt

Phenolic acids are wide spread phytochemicals, occurring naturally in many edible plants. They are known to exert several bioactivities, such as anti-inflammatory, anti-oxidant, and antibacterial [1,2]. However, their effect on the structure and dynamics of lipid bilayers is yet to be elucidated. This work aims to characterize phytochemical-membrane interactions, using both liposomes and a human cell line (RPE-1), through extensive fluorescence spectroscopy, both steady-state and time resolved.

The effect of rosmarinic acid (RA), an ester of caffeic acid (CA), was studied in different liposome models presenting each a different lipid phase behavior at 23°C [3], Liquid Disordered (L_d), Liquid Ordered (L_o) and L_d/L_o coexistence, respectively, the last being representative of the outer leaflet of human plasma membrane. Quercetin was also used as a positive control since its interactions with lipid bilayers are well characterized [4,5]. RA, CA and chlorogenic acid (CGA) have low lipophilicity [6] and among them, RA was the only with measurable partition to biological membrane models. Therefore, we proceeded to the evaluation of the effect of RA on several biophysical properties of the liposomes using two fluorescent probes with superficial membrane location, in the water/lipid interface, where phenolic acids are expected to localize [4,6]. RA effects were negligible even at high concentrations (400 μM), whereas quercetin, the positive control, had large effects at concentrations below 100 μM.

Living cells exposed to RA, CA and CGA are currently under study, in order to assess indirect repercussions of the phenolic acids on plasma membrane lipid bilayer, thus complementing the results obtained with the liposomes.

Acknowledgments: *FCT is acknowledged for CQB strategic project UID/Multi/00612/2013, and IF2012 initiative (POPH, Fundo Social Europeu).*

References

[1] Halliwell, B., Rafter, J. & Jenner, A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *1– 4. 81, 268–276* **(2005)**.

[2] Petersen, M. & Simmonds, M. S.. Rosmarinic acid. *Phytochemistry 62, 121–125* **(2003)**.

[3] de Almeida, R. F. M., Fedorov, A. & Prieto, M. Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts. *Biophys. J. 85, 2406–2416* **(2003)**.

[4] Marquês, J. T., Viana, A. S. & De Almeida, R. F. M. A biomimetic platform to study the interactions of bioelectroactive molecules with lipid nanodomains. *Langmuir 30, 12627–12637* **(2014)**.

[5] Kosinova, P., Berka, K., Wykes, M., Otyepka, M. & Trouillas, P. Positioning of antioxidant quercetin and its metabolites in lipid bilayer membranes: Implication for their lipid-peroxidation inhibition. *J. Phys. Chem. B 116, 1309–1318* **(2012)**.

[6] Hugo A. L. Filipe, Miguel Machuqueiro, Maria da Soledade Santos, Rodrigo F. M. de Almeida, unpublished work.

FC02 - HOW TO DISASSEMBLE A VIRUS CAPSID: A COMPUTATIONAL APPROACH

Claudio Alexandre Piedade[1], António E. N. Ferreira[1] and Carlos Cordeiro[1]

(1) *Laboratório de FTICR e Espectrometria de Massa Estrutural, Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.*

claudioalexandrepiedade@gmail.com

In contrast with the assembly process of virus particles, which has been the focus of many experimental and theoretical studies, the disassembly of virus protein capsids, a key event during infection, has generally been overlooked. Although the nature of the intracellular triggers that promote subunit disassembly may be diverse, here we postulate that the order of subunit removal is mainly determined by each virus structural geometry and the strength of subunit interactions. Following this assumption, we modelled the early stages of virus disassembly of T=1 icosahedral viruses, predicting the sequence of removal of up to five subunits in a sample of 51 structures. We used combinatorics and geometry, to find non-geometrically identical capsid fragments and estimated their energy by three different heuristics based on the number of weak inter-subunit contacts. We found a main disassembly pathway common to a large group of viruses consisting of the removal of a triangular trimer. Densoviruses lose a square-shaped tetramer while Human Adenoviruses lose a pentagon-shaped pentamer. Results were virtually independent of the heuristic measure used. These findings suggest that particular subunit interactions might be an important target for novel antiviral drugs designed to interfere with capsid disassembly.

Acknowledgments: *Work supported by project RECI/BBB-BEP/0104/2012 from Fundação para a Ciência e Tecnologia, Portugal.*

References

[1] Caspar, D. L. D. and Klug, A. (1962). *Physical Principles in the Construction of Regular Viruses.* Cold Spring Harbor Symposia on Quantitative Biology, 27(0):1–24.

[2] Castellanos, M., Prez, R., Carrillo, P., dePablo, P., and Mateu, M. (2012). *Mechanical Disassembly of Single Virus Particles Reveals Kinetic Intermediates Predicted by Theory.*

[3] Burnside, W. (1909). *Theory of groups of finite order*. Messenger of Mathematics, 23:112.

[4] Horton, N. and Lewis, M. (1992). *Calculation of the free energy of association for protein complexes.* Protein Science, 1(1):169–181.

[5] Ortega-Esteban, A., Prez-Bern, A. J., Menndez-Conejero, R., Flint, S. J., Martn, C. S., and de Pablo, P. J. (2013). *Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue.* Scientific Reports, 3.

[6] Hemberg M, Yaliraki SN, Barahona M. Stochastic Kinetics of Viral Capsid Assembly Based on Detailed Protein Structures. *Biophysical Journal*. 2006;90(9):3029-3042.

FC03 - UNTANGLING THE GENE NETWORKS FOR MOTOR NEURON DEGENERATION: FROM DISEASE MODEL TRANSCRIPTOMES TO CELLULAR SYSTEMS

Hugo A. F. Santos,[1] Takakazu Yokokura,[2] Seiko Yoshikawa,[2] Elizabeth McNeill,[3] Andreia Amaral,[4] David Van Vactor[2,3] and Margarida Gama-Carvalho[1]

(1) *University of Lisboa, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute Lisbon, Portugal;* ***(2)*** *Okinawa Institute of Science and Technology, Tancha, Onna, Japan;* ***(3)*** *Department of Cell Biology, Harvard Medical School, Boston, USA;* ***(4)*** *iMM - Instituto de Medicina Molecular, Lisbon, Portugal.*

hasantos@fc.ul.pt

Spinal Muscular Atrophy (SMA), a lethal neurodegenerative disorder, is characterized by low levels of the Survival of Motor Neuron (SMN) protein [1]. This protein is essential for the assembly of small nuclear ribonucleoproteins (snRNPs) [2], key components of the spliceosome - a large RNA-protein macromolecular complex in which splicing of pre-mRNA occurs [3]. Strikingly, even though efficient splicing is a basal requirement for every cell population, low levels of the ubiquitous SMN protein mainly affect motor neurons (MNs) [4].

Drosophila melanogaster models of SMA replicate the patient-observed neuromuscular junction phenotype and have allowed for a systematic screening of genetic modifiers of the disease [5]. Still, despite robust knowledge of SMA's genetics, the exact molecular mechanisms connecting SMN to MN degeneration remain elusive [1; 6].

We present a detailed analysis of the central nervous system transcriptome of a *Drosophila melanogaster* SMA model using high-depth RNA-Seq, providing novel insights into SMN-dependent changes in gene expression and their connection to MN degeneration. We further address the conservation of these mechanisms in human by correlating this data with the transcriptome profile of MN cell cultures derived from SMA patient fibroblasts. Our results identified conserved changes in the expression of genes revealing strong links to disease-relevant signalling pathways (e.g. BMP, FGF) previously described as modulators of the SMN-dependent loss-of-function phenotype [6]. We expect to pinpoint novel candidates for future complementary therapeutics, but more importantly we are dissecting the molecular mechanisms linking SMN down-regulation to specific gene expression changes underlying the SMA-associated neurodegeneration profile.

Acknowledgments: *The authors acknowledge the computational support kindly provided by Dennis Wall through the Sherlock cluster at Stanford School of Medicine and fruitful discussions with Javier de Las Rivas. Work supported by grants from FCT (UID/MULTI/04046/2013 and JPND-CD/0002/2013), NINDS (to DVV) and OIST. This is an EU Joint Programme - Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organisations under the aegis of JPND - www.jpnd.eu (France/ANR; Germany/BMBF; Portugal/FCT; Spain/ISCIII). Hugo A. F. Santos is a fellow of the BioSys-PhD programme at BioISI supported through the PD-F FCT programme (PhD Fellowship SFRH/BD/52492/2014).*

References

[1] Coovert, D. D., Le, T. L., McAndrew, P. E., Strasswimmer, J., Crawford, T. O., Mendell, J. R., Coulson, S. E., Androphy, E. J., Prior, T. W., Burghes, A. H. M. *Hum Mol Genet* 1997, *6* (*8*), 1205-1214; [2] Wan, L., Battle, D. J., Yong, J., Gubitz, A. K., Kolb, S. J., Wang, J., Dreyfuss, G. *Mol Cell Bio* 2005, *25,* 5543-5551; [3] Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., Steitz, J. A. *Nature* 1980, *283*, 220–224; [4] Burghes, A. H. M., Beattie, C. E. *Nat Rev Neurosci*. 2009, *10* (*8*), 597-609; [5] Sen, A., Dimlich, D. N., Guruharsha, K. G., Kankel, M. W., Hori, K., Yokokura, T., Brachat, S., Richardson, D., Loureiro, J., Sivasankaran, R., Curtis, D., Davidow, L. S., Rubin, L. L., Hart, A. C., Van Vactor, D., Artavanis-Tsakonas, S. *PNAS* 2013, *110* (*26*), 2371-2380; [6] Chang, H. C., Dimlich, D. N., Yokokura, T., Murkherjee, A., Kankel, M. W., Sen A., Sridhar, V., Fulga, T. A., Hart, A. C., Van Vactor D., Artavanis-Tsakonas, S. *PLOS One* 2008, *3* (*9*), e3209.

FC04 - THE NEURONAL S100B PROTEIN AS A NOVEL MODULATOR OF AMYLOID-β AGGREGATION IN ALZHEIMER'S DISEASE

Joana S. Cristóvão,[1] Vanessa Morris,[2] Els Pardon,[3] Jan Steyaert,[3]
Bernd Reif[2] and Cláudio M. Gomes[1]

(1) *Faculdade de Ciências, Biosystems and Integrative Sciences Institute and Department of Chemistry and Biochemistry, Universidade de Lisboa, Lisboa, Portugal.;* ***(2)*** *Technical University Munich, Munich, Germany;* ***(3)*** *Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan, Brussels, Belgium.*

jmcristovao@fc.ul.pt

Insoluble β-amyloid peptide (Aβ) deposits formed in the synaptic cleft and neuroinflammation are consistent features in Alzheimer's disease (AD) and strong candidates for the initiation of the neurodegeneration process. S100B is one of the most abundant pro-inflammatory proteins which is up regulated in AD and is found associated with senile plaques. S100B is a small dimeric protein whose structure and functional regulatory interactions with other proteins are modulated by calcium-binding through EF-hand motifs and by zinc-and copper-binding to the dimer interface. These facts and our recent observation that S100 proteins have intrinsic β-aggregation propensity [1,2] have prompted us to investigate the impact of S100B on Aβ fibrillation. Here we report the co-aggregation phenomena involving S100B and Aβ and the development of nanobodies targeting S100B. Our studies that combine biochemical, biophysical and structural approaches indicate that S100B is a new key modulator of Aβ42 aggregation. We found that S100B forms a complex with monomeric Aβ as shown by NMR and ITC, delaying the formation of ThT-binding Aβ oligomers, a finding corroborated by TEM imaging. Analysis of Aβ aggregation kinetics and subsequent data fitting elicited quantitatively the mechanisms involved. In parallel, we will report the characterization of a library of 20 nanobodies targeting S100B developed with the aim to generate biological tools to regulate the interaction between S100B and other proteins, such as Aβ42. With this approach, we expect to generate knowledge that will translate into the potential use of S100B as a new druggable target to prevent or ameliorate inflammation in neurodegeneration.

Acknowledgments: *This work was supported by Fundação para a Ciência e Tecnologia (FCT/MCTES, Portugal) through PhD fellowship SFRH/BD/101171/2014 (to JSC) and research grant PTDC/NEU-NMC/2138/2014 (to CMG); by Bial Foundation research grant 343/14 (to CMG) and by the EU-Instruct ESFRI project for the support and the use of shared resources in Structural Biology (fellowship to JSC).*

References

[1] Carvalho, S.B., et al., Intrinsically Disordered and Aggregation Prone Regions Underlie beta-Aggregation in S100 Proteins. PLoS One, 2013. 8(10): p. e76629.

[2] Carvalho, S.B., et al., Structural Heterogeneity and Bioimaging of S100 Amyloid Assemblies. Bionanoimaging: Protein Misfolding and Aggregation, 2013 DOI: 10.1016/B978-0-12-394431-3.00018-3.

FC05 - CFTR IN THE MAINTENANCE OF EPITHELIAL DIFFERENTIATION IN THE AIRWAYS

Margarida C. Quaresma,[1] Hugo M. Botelho[1] and Margarida D. Amaral[1]

(1) *University of Lisboa, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Campo Grande, C8, 1749-016 Lisboa, Portugal.*

mcquaresma@fc.ul.pt

Background: Cystic fibrosis (CF) is a multi-organ disease mostly affecting the airways. The disease is caused by mutations in the CFTR gene, encoding a protein expressed at the apical surface of epithelial cells. F508del, the most common CF-causing mutation, leads to impaired CFTR traffic to the plasma membrane.

There is significant evidence that CFTR expression levels and its plasma membrane traffic are deeply connected to the differentiation status of the cells. Lack of functional CFTR influences development (e.g. resulting in tracheal malformations), potentiates tissue fibrosis, and leads to impaired secretory cell and epithelial differentiation [1, 2, 3]. On the other hand, epithelial cell proliferation has been found to be upregulated in CF cells [4]. Consistently, CF patients are described to have an increased risk of cancer [5]. More recently, a dedifferentiation gene expression signature was found in the CF epithelium [6].

Objective: We aimed to assess whether absence of functional CFTR disrupts epithelial differentiation, using the airways as a model.

Methods: We optimized an airway cryopreservation and immunofluorescence (IF) protocol. Cryocuts of human CF (F508del/F508del) and non-CF airway tissue were characterized by IF regarding expression and localization of a panel of epithelial/mesenchymal markers (e.g. E- and N-cadherin, KI67, CFTR).

Results and Discussion: IF evidenced a downregulation in the differentiation and an upregulation in the dedifferentiation markers in CF lung tissue. A proliferation marker (KI67) also showed increased expression in the CF epithelia. Overall, the data supports an association between CFTR dysfunction and perturbed airway differentiation pathways.

Acknowledgments: *Work supported by UID/MULTI/04046/2013 centre grant from FCT, Portugal (to BioISI) and FCT/POCTI (PTDC/BIM-MEC/2131/2014) "DIFFTARGET" (to MDA). MCQ is a recipient of a fellowship from BioSys PhD programme (Ref PD/BD/114389/2016) and HMB of a post-doctoral fellowship (SFRH/BPD/93017/2013) both from FCT (Portugal).*

References

[1] Meyerholz et al, *Am. J. Respir. Crit. Care Med.* **2010**, 182, 1251–1261.

[2] Larson et al, *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2000**, 279, L333–L341.

[3] Hajj et al, *J. Pathol.* **2007**, 211, 340–350.

[4] Leigh et al, *Am. J. Respir. Cell Mol. Biol.* **1995**, 12, 605–612.

[5] Maisonneuve et al, *J. Natl. Cancer Inst.* **2013**, 105, 122–129.

[6] Clarke et al, *Genomics* **2015**, 106, 268–277.

Chemistry
Flash Communication
Abstracts

FC06 - CHEMICAL AND METROLOGICAL CHALLENGES TO THE QUALITY OF SEAWATER

Bárbara Anes,[1] Cristina Oliveira,[1] Ricardo Bettencourt da Silva[1] and Maria Filomena Camões[1]

(1) *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Portugal.*

bvanes@fc.ul.pt

Increasing concentrations of CO_2 in the atmosphere are followed by dissolution in seawater with consequent acidification, making the complex carbonate system in the oceans the most important one.

To detect small changes in CO_2, it is necessary to measure the system master variables: pH, alkalinity, pCO_2 and DIC. Knowing two of these variables makes it possible to calculate the concentrations of H_2CO_3 or CO_3^{2-} that cannot be measured directly [1].

Free acidity, expressed by pH, is an important parameter for the characterization of seawater and assessment of ocean acidification that affects the biogeochemical processes in the ocean. However, in order to measure pH of seawater - a complex matrix with high ionic strength ($I \approx 0.67$ mol kg^{-1}), appropriate calibration buffer solutions have to be adopted [2]. This has led to the development of methods and procedures for measuring pH in seawater with the lowest possible uncertainty [3] enabling detection of small variations in space, time and between laboratories, thus allowing to overcome problems associated with lack of comparability of the values obtained by the oceanographic community.

This research work includes the evaluation of reliable carbonic acid ionization constants in seawater with their associated uncertainties, which remains a metrological challenge.

Acknowledgments: *This work was financially supported by the EMRP- ENV 05 OCEAN and the associated REG2. The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union. The authors wish to acknowledge the Portuguese funding institution Fundação para a Ciência e a Tecnologia - FCT, for supporting their research, namely through SFRH/BD/111437/2015 grant and UID/QUI/00100/2013 project.*

References

[1] W. Stumm, J.Morgan, *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*. 3rd Edition. **1995**, New York: Wiley.

[2] R.P. Buck, S. Rondinini, A.K. Covington, F.G.K. Baucke, C.M.A. Brett, M.F. Camoes, M.J.T. Milton, T. Mussini, R. Naumann, K.W.Pratt, P. Spitzer, G.S.Wilson, Pure and Applied Chemistry **2002**, 74, 2169-2200.

[3] B. Anes, R.J.B. Silva, H. F. P. Martins, C. Oliveira, M. F. Camões, Accred. Qual. Assur. **2015**; DOI 10.1007/s00769-015-1163-y.

FC07 - IMMOBILIZATION OF LACCASE AND MAGNETITE NANOPARTICLES ON A BIOCOMPATIBLE POLYMER FOR ELECTROCHEMICAL BIOSENSING

Luís C. Almeida,[1] M. D. Carvalho,[1] J. P. Correia[1] and A. S. Viana[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.*

luismdealmeid@gmail.com

The development of electrochemical biosensors, trough the immobilization of enzymes in electrode surfaces, is a promising field to achieve portable, fast and reliable devices needed for chemical analysis in the sampling site. [1] To overcome problems regarding stability of immobilized enzymes, bio-inspired materials such as polydopamine films (PDA) are been explored to covalently bind target biomolecules though the latent reactivity of quinone groups. [2] Furthermore, notorious catalytic properties of enzymes when conjugated with metal [3] and metal oxide nanoparticles [4] was recently reported by our group.

This work addresses the optimization of catalytic activity of immobilized laccase (phenol oxidase) in carbon electrodes modified with PDA films and magnetite type nanoparticles ($Fe_{3-x}O_4$). An extensive study of spontaneously formed PDA films properties was carried out in glassy carbon electrodes by ellipsometry, water contact angles, atomic force microscopy and cyclic voltammetry. Preliminary studies of electrosynthesized PDA were also carried out. The catalytic performance of the enzyme nanostructured electrodes was assessed by chronoamperometry and cyclic voltammetry toward the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid diammonium salt (ABTS). Significant improvements on enzyme kinetics and electrode sensitivity were observed when a simple one-step methodology to co-immobilize the catalytic components was used. The results reveal a great potential application of this electrode architecture in novel biosensing devices.

Acknowledgments: *The authors are grateful to Professor Alessandra Morana, Francesco Cara and Guiseppe Squillaci (Institute of Agro-environmental and Forest Biology, Naples, Italy) for providing and characterizing Laccase. The work was financially supported by FCT, through projects: PEst 2015-2020-UID/Multi/00612/2013, IF/00808/2013 (POPH, UE-FSE) and PTDC/CTM-NAN/0994/2014.*

References

[1] S. Alegret, A. Merkoçi, *Comprehensive Analytical Chemistry - Electrochemical Sensor Analysis*, Elsevier, Netherlands, **2006**. [2] Y. Lui, K. Ai, L. Lu, *Chem. Rev.* **2014**, 114, 5077-5115.

[3] I. Almeida, V.C. Ferreira, M.F. Montemor, L.M. Abrantes, A.S. Viana, *Electrochimica Acta* **2012**, 83, 311–320.

[4] I. Almeida, F. Henriques, M.D. Carvalho, A.S. Viana, *J. Coll. Int. Sci.* **2017**, 485, 242-250.

FC08 - NON-RELEASING ANTIFOULING COATINGS: AN ECO-FRIENDLY STRATEGY FOR SURFACES PROTECTION AGAINST BIOFOULING

O. Ferreira,[1, 2] J.C. M. Bordado[2], M. J. Calhorda[1] and E. R. Silva [1, 2]

(1) *Departamento de Engª Química, Instituto Superior Técnico, Universidade Técnica de Lisboa, Avenida Rovisco Pais, 1049-001 Lisboa, Portugal;* ***(2)*** *Centro de Química e Bioquímica/Departamento de Química e Bioquímica (CQB/DQB) Universidade de Lisboa, Faculdade de Ciências, Campo Grande, 1749-016, Lisboa, Portugal*

Olga.ferreira@gmail.com

Biofouling, known as the adhesion of micro and macro-organisms on submersed surfaces, is one of most important factors that affect the efficiency of waterborne systems (seawater/fresh water). It is the cause of serious detrimental effects on such surfaces leading to subsequent economic and environmental penalties. It can be, for instance, the cause of hydrodynamic drag increasing in ships and thereby fuel consumption and greenhouse gas (SOx, NOx, CO_2) emissions [1]. Protection surface strategies against such biofouling have been widely pursued [2, 3]. Hitherto, antifouling biocide-releasing coatings seem to be the most effective, but the biocides ecotoxicity has led to strict regulations for their use, and those expected to come in 2013 will restrict even further the antifouling biocides currently in use. Therefore, greener antifouling alternatives are sought. In this work, a new approach for non-release antifouling coatings is being pursued [4]. It consists of the modification/functionalization of already proved biocides (e.g. commercially available) or other potential antifouling compounds in order to chemically immobilize them into conventional coatings' systems. The first task of this work involved the identification and immobilization of potential antifouling compounds in different polymer matrix (e.g. polyurethane/silicone matrices). Bacteriologic analysis of the modified biocides revealed that they still possess its biological activity. Antifouling assessment of the developed antifouling coatings under simulated/real field tests (ships, aquaculture nets and PVC prototypes) evidenced promising behaviours. At real field conditions silicone based coatings remain relative clean for more than a year.

Acknowledgments: *E. R. Silva is grateful for a research grant provided by Fundação para a Ciência e a Tecnologia (FCT) (SFRH/BPD / 88135 / 2012). The authors also thank the financial support provided from the FP7 FOUL-X-SPEL and Promar (Programa Operacional Pesca 2007-2013) projects by European Commission, and FCT (PEst-OE/QUI/UI0612/2011).*

References

[1] International maritime Organization, MEPC 59/INF.10.2009.

[2] I. Banerjee, R. C. Pangule, R. S. Kane, *Adv. Mat.* **2011**, 23, 690- 718.

[3] J. Chan, S. Wong in *Biofouling Types, Impact and Anti-Fouling*, Pollution Science, Technology and Abatement Series, Nova Science Publishers, Inc. New York, 2009.

[4] *E.R. Silva, O. Ferreira, J.C. Bordado, Patent Application WO2016/093719 A1,* ***2016****.*

FC09 - IDENTIFICATION OF CITALOPRAM TRANSFORMATION PRODUCTS BY LC QTOF-MS

Rodrigo A. Osawa,[1,2] Maria Helena Florêncio[1] and Maria da Conceição Oliveira[3]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, ULisboa, Portugal;* ***(2)*** *CAPES - Ministro da Educação do Brasil, Brasil;* ***(3)*** *Centro de Quimica Estrutural, Instituto Superior Técnico, ULisboa, Portugal.*

rodrigo.osawa@gmail.com

Citalopram, a selective serotonin reuptake antidepressant inhibitor (SSRI), has a high consumption in the world for the treatment of depression [1]. There are numerous studies that have detected the drug in effluents and surface waters [2,3] but there are few studies of the fate and transformation products (TPs) in the environment or in the wastewater treatment plant (WWTP).

The objective in this study is to identify the formation of TPs of citalopram that might be found in the environment by means of simulations of processes that may occur in the aquatic environment and in the wastewater treatment plant: hydrolysis; photolysis under ultraviolet (UV) irradiation and chlorination. The TPs were identified by liquid chromatography quadrupole-time-of-flight mass spectrometry (LC QTOF-MS), superficial water samples spiked with citalopram were analyzed in full-scan mode and in broadband collision-induced dissociation (bbCID) acquisition mode. The injection was performed in positive and negative ion mode. The experiments resulted in 15 possible identified TPs: 4 from hydrolysis; 3 from photolysis, 8 from chlorination, but some TPs were formed in more than one degradation process. The probable structures of TPs were established based on two prediction tools softwares: EAWAG-BBD: Pathway Prediction and Bruker MetabolitePredict. Analyses were based on accurate mass and on the fragmentation observed in the MS spectra. A possible degradation pathway was proposed for the formations of TPs and the stability and formation of TPs was monitored in the experiments.

Acknowledgments: *This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for PhD grants (BEX 0845-14-0) Fundação para a Ciência e a Tecnologia (FCT), Portugal (Projects UID/MULTI/00612/2013).*

References

[1] OECD. Health at a Glance: Europe 2012. OECD Publishing (2012).

[2] Evgenidou, E. N. et al., Science of the Total Environment 505 (2015), p.905–926.

[3] Subedi, B.; Kannan, K. Science of the Total Environment 514 (2015), p.273–280.

FC10 - A CAPTURE OF SMALL MOLECULES BY METAL-ORGANIC STRUCTURES

S. Realista,[1] J. C. Almeida, [1] S. Barroso,[2] N. A. G. Bandeira,[1] M. J. Calhorda[1] and P. N. Martinho[1]

(1) *Centro de Química e Bioquímica, DQB, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;*
(2) *Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais, 1049-001 Lisboa, Portugal*

smrealista@fc.ul.pt

Host-guest structures have proved to be useful for the recognition and activation of small molecules [1]. Cascade complexes with polyaza ligands have demonstrated ability to bind different small molecules by adapting their binding sites towards these molecules. Nelson's cryptands [2] are an example of a dynamic structure with useful applications, which demonstrated the ability to capture and convert CO2 to carbonate and methyl carbonate following its coordination to encapsulated metal ions. Here we explore the fixation chemistry of small molecules by derivatised dinuclear Cu(II), Ni(II) cryptands (**Figure 1**) where the phenyl ring was modified towards engineering these metal-organic structures into supramolecular assemblies. Attaching electron withdrawing or electron donating groups to the phenyl ring proved to affect their ability to capture CO2. Synthesis of the cryptates were performed under N2 and CO2 atmosphere, to understand the substituent effect, DFT studies were performed, and their behaviour was studied by cyclic voltammetry.

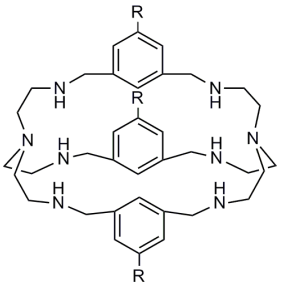


Figure 1. Derivatised cryptands.

Acknowledgments: *The authors thank FCT for financial support UID/MULTI/00612/2013 and PTDC/QEQ-QIN/3414/2014. PNM and SR thanks FCT and CATSUS Doctoral programme for financial support (SFRH/BPD/73345/2010 and PD/BD/52368/2013).*

References

[1] D-C Zhongab, T-B Lu, *Chem. Comm.,* **2016**, *52,* 10322-10337.

[2] Y. Dussart, C. Harding, P. Dalgaard, C. McKenzie, R.Kadirvelraj, V.Mckee, J. Nelson, *J. Chem. Soc. Dalton Trans.,* **2002**, 1704-1713.

Poster Communication Abstracts

P01 - LYSOSOMAL-MIMICKING VESICLES AS A TOOL TO STUDY THE EFFECT OF SPHINGOSINE ABNORMAL ACCUMULATION ON MEMBRANE BIOPHYSICAL PROPERTIES

Ana C. Carreira,[1,2] Rodrigo F.M. de Almeida[2] and Liana C. Silva[1]

(1) *iMed.ULisboa –Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa,1649-003 Lisboa, Portuga;* ***(2)*** *Centro de Química e Bioquímica, DQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.*

anacarreira@ff.ulisboa.pt

Artificial membranes have been widely used to unveil specific lipid-lipid and lipid-protein interactions in complex cellular systems. In this work, we developed a new synthetic biosystem that more closely resembles the lysosome – the lysosome-mimicking vesicles (LMVs), displaying internal acidic pH and external neutral pH. The LMVs were used to further understand how the sphingosine (Sph) abnormal accumulation in Niemann Pick type C1 (NPC1) impacts lysosomal membrane structure and biophysical properties. To this end, ternary 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/Sphingomyelin (SM)/Cholesterol (Chol) mixtures with, respectively, low and high Chol/SM levels (NPC1 phenotype) were prepared. The effect of Sph on the membrane permeability and biophysical properties was then evaluated by fluorescence spectroscopy, electrophoretic and dynamic light scattering. Our results showed that Sph has the ability to cause a shift in vesicle surface charge, increase the packing properties of the membrane and promote a rapid increase in membrane permeability. These effects are enhanced in NPC1-LMVs, i.e., containing higher levels of Chol and SM. Overall, the results suggest that lysosomal accumulation of these lipids, as observed under pathological conditions, might significantly affect lysosomal membrane structure and integrity, and therefore disturb cellular homeostasis.

Acknowledgments: *This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal: PTDC/BBB-BQB/0506/2012, PTDC/BBB-BQB/3710/2014, UID/00612/2013, SFRH/BD/88194/2012 to ACC and Investigador FCT to RFMA and LCS.*

P02 - IMPORTANCE OF ANOCTAMINS FOR CALCIUM SIGNALLING IN DIFFERENT CELLULAR LOCALIZATIONS

Ana Fonseca,[1,2] Karl Kunzelmann,[1] Margarida Amaral,[2] Rainer Schreiber[1] and Inês Cabrita[1]

(1) *Department of Physiology, University of Regensburg, Germany;* ***(2)*** *Departamento de Química e Bioquímica, Faculdade de Ciências Universidade de Lisboa, Portugal.*

ana.fonseca93@hotmail.com

Anoctamins (ANO/TMEM16) are a family of Calcium (Ca^{2+})-activated Chloride (Cl^-) Channels (CaCCs), which function is not yet fully disclosed. Involved in physiological processes like contraction and secretion, this channels might also modulate local Ca^{2+} signalling [1]. Interestingly, ANO1 was found to facilitate [Ca^{2+}] increase [2] and to tether the endoplasmic reticulum (ER) to the plasma membrane (PM) [3]. Remarkably, ANOs were found in a cellular compartment called primary cilium [4,5], a sensory organelle involved in Ca^{2+} signalling. Still, it remains unclear how ANOs change Ca^{2+} in specific microdomains. Therefore, two domain targeting Ca^{2+} indicators were used to study the ANO function in compartmentalized Ca^{2+} signalling. ANO1 and ANO4 overexpressing cells were measured with a cytosolic Ca^{2+} dye (Fura-2) and with a PM-targeted Ca^{2+} indicator (PM-GCaMP2). ANO1 increased the ATP-induced [Ca^{2+}] response near the PM, whereas ANO4 decreased the intracellular [Ca^{2+}]. Additionally, cell lines stably expressing ciliary-targeting Ca^{2+} indicators [6] were developed and the ciliary Δ[Ca^{2+}] were measured upon treatment with different channel inhibitors. Immunostaining revealed that the sensor is present in the primary cilium, but also in the PM, enabling the detection of simultaneous ciliary and PM Δ[Ca^{2+}]. However, the inhibitors caused unexpected Ca^{2+} increases that difficult clear conclusions. This results support a function for ANOs as Ca^{2+} modulators in microdomains, where ANO1 might act as a tethering protein increasing Ca^{2+} on the PM, while ANO4 might induce Ca^{2+} leakage from the ER. In the primary cilium their role is still uncertain and it could be further studied by ANO silencing.

Acknowledgments: *This work was supported by the University of Regensburg, DFG SFB699 funding. 5HT6-G-GECO1.0 was a gift from Takanari Inoue (Addgene plasmid # 47499). We thank Dr. J. Brunner, Department of Biochemistry, University of Zurich, Switzerland, for providing the nhTMEM16 cDNA.*

References

[1] K. Kunzelmann, I. Cabrita, P. Wanitchakool, J. Ousingsawat, L. Sirianant, R. Benedetto, R. Schreiber, Pflugers Arch. Eur. J. Physiol. 468 (2016) 475–490.

[2] R. Schreiber, D. Faria, B. V. Skryabin, P. Wanitchakool, J.R. Rock, K. Kunzelmann, Pflugers Arch. Eur. J. Physiol. 467 (2014) 1203–1213.

[3] X. Jin, S. Shah, Y. Liu, H. Zhang, M. Lees, Z. Fu, J.D. Lippiat, D.J. Beech, A. Sivaprasadarao, S. a Baldwin, H. Zhang, N. Gamper, Sci. Signal. 6 (2013) ra73.

[4] R. Schreiber, K. Kunzelmann, Pflügers Arch. - Eur. J. Physiol. 1 (2016).

[5] V. Forschbach, M. Goppelt-Struebe, K. Kunzelmann, R. Schreiber, R. Piedagnel, a Kraus, K.-U. Eckardt, B. Buchholz, Cell Death Dis. 6 (2015) e1899.

[6] S. Su, S.C. Phua, R. DeRose, S. Chiba, K. Narita, P.N. Kalugin, T. Katada, K. Kontani, S. Takeda, T. Inoue, Nat. Methods 10 (2013) 1105–7.

P03 - REGULATION OF GLUCOSE UPTAKE IN MAMMALIAN CELLS BY PROTEIN PHOSPHORYLATION

Andreia Almeida Henriques,[1,3] Luka Clarke[1,2] and Peter Jordan[1,3]

(1) *BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal ;* ***(2)*** *Biochemistry Department, Faculty of Sciences, University of Lisbon, Lisbon, Portugal;* ***(3)*** *Department of Human Genetics, National Health Institute Dr. Ricardo Jorge, Lisbon, Portugal.*

afhenriques@fc.ul.pt

Cancer cells demand increased amounts of glucose to sustain their proliferation and upregulate plasma membrane expression of glucose transporter GLUT1. In insulin responsive cells, glucose uptake requires previous phosphorylation of TBC1D4, a negative regulator of endosomal GLUT traffic. Previous work published by the host lab [1] has discovered that protein kinase WNK1 can also phosphorylate TBC1D4 and promote the translocation of GLUT1 to the cell surface. In vitro, WNK1 also phosphorylates the homologue TBC1D1 for which a role in cancer cell glucose uptake is not known. The extent to which WNK1 and both TBC1D proteins contribute to glucose uptake in cancer cells is not understood but its characterization is required for a systems-based understanding of glucose metabolism. In order to characterize the role of protein kinase WNK1, various colorectal cancer cell lines were first cultivated in the presence of different glucose concentrations. The amount of GLUT1 at the cell surface was compared under these conditions and the effect of depleting WNK1 expression by siRNA determined. For selected conditions, key cell cycle or apoptotic marker proteins were analyzed by Western blot in order to relate the role of WNK1 glucose-dependent cell growth and survival. WNK1-depleted cells cultured in low glucose medium showed higher apoptotic and cell-cycle arrest phenotypes.
Concerning the key phosphorylation events involved in the regulation of GLUT1, mass spectrometry analysis revealed the WNK1-specific phosphorylation of TBC1D1-Ser565 and TBC1D4-Ser704. Phospho-mimetic mutants TBC1D4-Ser704D and TBC1D1-Ser565D will be tested for their ability to increase GLUT1 translocation.

Acknowledgments: *Support for this work was provided by FCT and BioISI with the scholarship PD BD 106080/2015.*

References

[1] Mendes AI, Matos P, Moniz S, Jordan P. Protein kinase WNK1 promotes cell surface expression of glucose transporter GLUT1 by regulating a Tre-2/USP6-BUB2-Cdc16 domain family member 4 (TBC1D4)-Rab8A complex. J Biol Chem **2010** 285:39117-39126.

P04 - *IN VITRO* CHARACTERIZATION AND CORRECTION OF SPLICING MUTATIONS IN IVS 5 BY AN ANTISENSE OLIGONUCLEOTIDE

Verónica Felício,[1] Arsénia Massinga,[1] Nikhil T. Awatade,[1] Luka A. Clarke,[1] Susana Igreja[1] and Margarida D. Amaral[1]

(1) *University of Lisboa, Faculty of Sciences, BioISI-Biosystems and Integrative Sciences Institute, Lisboa, Portugal*

ajmassinga@fc.ul.pt

Background: Until now, more than 2,000 alterations have been described in the CFTR gene, most presumed to be pathogenic [1]. A significant fraction of these (~11%) affects pre-mRNA splicing. Novel therapeutic approaches to correct splicing mutations have been described using antisense oligonucleotides (AONs) [2]. We have recently reported a novel splicing mutation - 711+3A>T in IVS5 – found in a CF patient [3].

Objective: Herein, we aim to correct the aberrant splicing caused by 711+1G>T, 711+3A>T, 711+3A>G and 711+5G>A using an RNA-based AON strategy.

Methods: To this end, we designed one single AON complementary to the pre-mRNA area of interest in IVS5 and tested its effect in the above splicing mutations using a mini-gene consisting in full-length CFTR cDNA plus the intronic regions IVS4 and IVS5. The correction of splicing was assessed by quantitative RT-PCR (qRT-PCR).

Results and Discussion: Our qRT-PCR data show that all the above splicing mutations lead to skipping of IVS5 and very little production of wt transcripts, namely: 21% (711+3A>T), 2% (711+1G>T), 9% (711+3A>G) and 11% (711+5G>A). Our data also show that for all the splicing mutations the AON significantly restored exon 5 inclusion in CFTR mRNA in cells transfected with this AON, namely to the following levels: 52%, 12%, 19% and 50%, respectively.

In conclusion, our *in vitro* studies revealed that four splicing mutations in IVS5, lead to aberrant transcripts. However, exon skipping can be corrected by a single AON, thus suggesting that this AON has some therapeutic potential for CF patients carrying any of these mutations.

Acknowledgments: *Work supported by UID/MULTI/04046/2013 centre grant from FCT, Portugal (to BioISI) and Gilead GÉNESE Programme grant Ref PGG/008/2015 (to MDA). VF and NTA are recipients of PhD fellowships (SFRH/BD/87478/2012 and SFRH/PD/BD/52487/2014 respectively, the latter from BioSys PhD programme) and SI by Post-doctoral fellowship (SFRH/BPD/85363/2012) all from FCT, PortugalT.*

References

[1] http://www.genet.sickkids.on.ca/StatisticsPage.html

[2] S. Igreja, *et. al*. *Hum Mutat*. **2015**, 37, 209-215.

[3] V. Felício, *et. al. Clin Genet*. **2016**, doi: 10.1111/cge.12802.

P05 - LIQUID ORDERED PHASE FORMATION BY PLASMA MEMBRANE STEROLS ESTABLISHES A CLOSE PARALLEL BETWEEN MOLECULAR AND CELLULAR BIOPHYSICS

Catarina A. C. Antunes,[1] Alena Khmelinskaia,[1,#] André E.P. Bastos,[1] Gerson M. da S. Lobo,[1] Rui Malhó,[2] L. Cyrne,[1] H. Susana Marinho[1] and Rodrigo F. M. de Almeida[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *BioISI, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(#)*** *current address: Max-Planck Institut for Biochemistry, Department of Cellular and Molecular Biophysics, 82152 Planegg, Germany*

catarina.alves.antunes@gmail.com

Ergosterol (erg) and cholesterol (chol) are the major sterols in the plasma membrane of fungi and mammalians, respectively. They share several metabolic precursors, including zymosterol (zym), the major sterol in *erg6*Δ yeast mutant cells. To understand the relevance of sterol structure on the formation and properties of plasma membrane microdomains in eukaryotes, detailed biophysical studies were conducted. It was shown in liposomes that chol and erg interact more strongly with saturated than unsaturated glycerophospholipids, whereas zym interacts similarly with both. These selective interactions help explaining the ability for chol and erg to form liquid ordered (l_o) raft-like domains in opposition to zym. The same behaviour was observed when evaluating lipid bilayer passive permeability to water and univalent cations. The presence of zym, unlike erg or chol, does not alter passive permeability. These results validate that zym does not affect the gel/liquid disordered (l_d) domains or their coexistence, responsible for most of the permeation registered. However, erg and chol change the gel/l_d into a less permeable l_o/l_d coexistence, as observed in giant liposomes by confocal microscopy.

The distribution heterogeneity at the plasma membrane of Can1p-GFP, a protein marker of erg-enriched domains, was similar in wild-type and *erg6*Δ despite a higher level of expression in the mutant cells, possibly to compensate for traffic defects at the Golgi complex. Hence, the inability of zym to promote the formation of l_o-like domains observed in our *in vitro* studies provide a biophysical explanation for the results obtained *in vivo*, and gives support to l_o formation as a convergent evolutionary feature in eukaryotes.

Acknowledgements: *Fundação para a Ciência e a Tecnologia (FCT), Portugal is acknowledged for grants PTDC/BBB-BQB/6071/2014 and UID/Multi/00612/2013, IF2012 initiative (P.O.P.H., F.S.E.), and Ph.D. fellowship SFRH/BD/88199/2012 to A.E.P.B. We thank Prof. W. Tanner (University of Regensburg, Germany) for the kind gift of Plasmid Ylp211, which was transformed into BY4741 (wt) strain to obtain strain LC4 expressing CAN1-GFP, and Prof. A. Herrmann and Dr. S. Scolari (Humboldt University, Berlin, Germany) for FLIM studies and scientific insights.*

P06 - REGULATION OF EPITHELIAL CHLORIDE TRANSPORT BY PHOSPHO-TYROSINE-INITIATED PROTEIN NETWORKS

Cláudia Loureiro[1,2,3], Luka Clarke[2,3] and Peter Jordan[1,3]

(1) Department of Human Genetics, National Health Institute Doutor Ricardo Jorge (INSA), Lisbon; ***(2)*** Biosystems & Integrative Sciences Institute (BioISI), Lisbon; ***(3)*** Faculty of Sciences, University of Lisboa, Lisbon.
loualmclaudia@gmail.com

Protein kinase Syk was recently found to phosphorylate a specific tyrosine residue in three distinct disease-related chloride transport proteins: CFTR (in cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD)), NKCC2 and KCC3 (in kidney function and hypertension). Tyrosine phosphorylation downregulates the amount of CFTR [1] present at the plasma membrane and a better understanding of this process may reveal novel therapeutic options for CF patients. Thus, we determined the adaptor proteins containing phospho-tyrosine-binding domains involved in the process.

For their identification, we used biotinylated synthetic peptides containing the respective CFTR, NKCC2 or KCC3 phospho-tyrosines as baits and isolated adaptor proteins from physiologically relevant human or mouse cell lysates. After the peptide pull-down the samples were sent for mass spectrometry by Nano-LC-Triple TOF analysis to identify the obtained complex mixture of proteins. Following a statistical analysis in order to choose the best candidates for experimental validation, we identified some proteins with phosphor-tyrosine-binding domains together with proteins potentially involved in membrane traffic. Currently, we are validating candidate proteins chosen using the peptide pull-down and recombinant transporter fragments and measuring the effect of adaptor protein depletion on the amount of chloride transport proteins at the plasma membrane.

The results are expected to reveal the underlying molecular mechanisms and suggest novel therapeutic targets for diseases like CF, COPD or hypertension.

Acknowledgments: *Funding Support by FCT (PD/BD/52488/2014).*

References

[1] Mendes et al., *Mol Cell.Biol.* **2011**, *31,* 4076-4086.

P07 - ROLE OF CFTR AND ANO6 FOR ROS-MEDIATED APOPTOSIS

Filipa Simões,[1] Jiraporn Ousingsawat,[1] Podchanart Wanitchakool,[1] Ana Fonseca,[1] Rainer Schreiber[1] and Karl Kunzelmann[1]

(1) *University of Regensburg, Institute of Physiology, Regensburg, Germany*

fbsimoes@fc.ul.pt

Cystic fibrosis (CF) is the most common autosomal recessive disease found in Caucasians. It is caused by mutations in the CFTR gene, which encodes for a Cl^- channel responsible to maintain ion and fluid homeostasis in the intestine, pancreas, airways and sweat glands [1]. CF is characterized by an apoptotic dysfunction, which may contribute for the ongoing inflammations found in this disease [2]. In this regard, studies have shown that CFTR controls the intracellular redox status, acidification and ceramide content in lipid rafts. Interestingly, CFTR is reported to interact with other Cl^- channels, such as Anoctamin 6 (ANO6, TMEM16F) [3,4]. This a phospholipid scramblase involved in apoptosis that mediates PS exposure in the outer membrane leaflet, leading to the engulfment of the dying cell by phagocytes [5]. Thus, it was asked if ANO6 and CFTR share a functional relationship during ROS-mediated apoptosis. Using different *in vitro* systems, it was concluded that ANO6 is activated by ROS as an ion channel and a phospholipid scramblase, being involved in cell shrinkage and PS exposure. Moreover, CFTR was also found to contribute for cell death independently of pore opening and channel stimulation. Co-expression of ANO6 and CFTR resulted in an increase of both spontaneous and ROS-induced apoptosis. Taken together, exposure of cells to ROS leads to mitochondrial permeabilization, release of Ca^{2+} and pro-apoptotic proteins, responsible for caspase cleavage. These events terminate with ANO6 activation, which may support cell shrinkage and phospholipid scrambling, two apoptotic hallmarks enhanced in the presence of CFTR.

References

[1] Cutting, G. R. Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat. Rev. Genet.* **16,** 45–56 (2015).

[2] Soleti, R., Porro, C. & Martı, M. C. Apoptotic process in cystic fibrosis cells. 1029–1038 doi:10.1007/s10495-013-0874-y

[3] Martins, J. R. *et al.* Anoctamin 6 is an essential component of the outwardly rectifying chloride channel. *Proc. Natl. Acad. Sci. U. S. A.* **108,** 18168–72 (2011).

[4] Tobergte, D. R. & Curtis, S. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. *J. Chem. Inf. Model.* **53,** 1689–1699 (2013).

[5] Suzuki, J. & Nagata, S. Phospholipid scrambling by TMEM16F. *Seikagaku* **83,** 1050–1054 (2011).

P08 - SPHINGOLIPID-ENRICHED DOMAINS EVOLVE DURING CONIDIAL GROWTH

Filipa C. Santos,[1] Andreia S. Fernandes,[2,3] Catarina A. C. Antunes,[1] Filipe P. Moreira,[1] Arnaldo Videira,[2,3,4] H. Susana Marinho[1] and Rodrigo F. M. de Almeida[1]

(1) *CQB, DQB, FCUL, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *IBMC, Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal;* ***(3)*** *i3S, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal,* ***(4)*** *ICBAS, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal*

fpsantos@fc.ul.pt

Neurospora crassa, a haploid ascomycete, in the unicellular conidial stage has ideal features to study sphingolipid (SL)-enriched domains [1] and has been used as a biological model for multicellular eukaryotes, in the study of developmental processes, apoptosis [2], aging [3] and in the molecular basis of the circadian rhythm observed in asexual spore formation, conidiation [4]. Several changes in lipid metabolism and in the membrane composition of *N. crassa* occur during spore germination. However, the biophysical impact of those changes is unknown. Hence, a biophysical study of *N. crassa* plasma membrane (PM), particularly SL-enriched domains, and their dynamics along conidial growth is prompted.

Two fungal strains, wild-type (WT) and slime, which is devoid of cell wall, were studied. Conidial growth of WT from a dormancy state to an exponential phase was accompanied by membrane reorganization, namely an increase of membrane fluidity, occurring faster in a supplemented medium than in Vogel's minimal medium. Gel-like domains, likely enriched in SLs, were found in both *N. crassa* strains, but were particularly compact, rigid and abundant in the case of slime cells, even more than in budding yeast *Saccharomyces cerevisiae*. In *N. crassa*, our results suggest that the melting of SL-enriched domains occurs near growth temperature (30 ºC) for WT, but at higher temperatures for slime. Regarding biophysical properties strongly affected by ergosterol, the PM of slime conidia lays in between those of *N. crassa* WT and *S. cerevisiae* cells. The differences in biophysical properties found in this work, and the relationships established between membrane lipid composition and dynamics, give new insights about the PM organization and structure of *N. crassa* strains during conidial growth [5].

Acknowledgments: *FCT is acknowledged for F.C.S. Ph.D. scholarship (SFRH/BD/108031/2015), research grants PTDC/BBB-BQB/6071/2014, including a fellowship to C.A.C.A., UID/Multi/00612/2013, IF2012 initiative (POPH, Fundo Social Europeu) and PT2020 referring to research unit 4293.*

References

[1] D.P. Beck, J.W. Greenawalt, *J. Bacteriol.* ***1977***, *131*, 188-193.

[2] A.S. Fernandes, A. Castro, A. Videira, *Apoptosis* ***2013***, *18,* 940-948.

[3] M.E. Case, J. Griffith, W. Dong, I.L. Tigner, K. Gaines, J.C. Jiang, S.M. Jazwinski, J. Arnold, *Ecol Evol* ***2014*****,** *4,* 3494-3507.

[4] H. Nakashima, K. Onai, *Sem Cell Dev Biol* ***1996*****,** *7*, 765-774.

[5] F.C. Santos, A.S. Fernandes, C.A.C. Antunes, F.P. Moreira, A. Videira, H.S. Marinho, R.F.M. de Almeida, *BBA-Mol Cell Biol Lipids* ***2017***, *1862*, 156-166.

P09 - COMPLEX SPHINGOLIPIDS FROM SACCHAROMYCES CEREVISIAE: EXTRACTION, ANALYSIS, AND PRELIMINARY BIOPHYSICAL CHARACTERIZATION IN VIVO

Filipe P. Moreira,[1] Catarina A. C. Antunes,[1] H. Susana Marinho[1] and Rodrigo F.M. de Almeida[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*
filipepalatino.moreira@gmail.com

The plasma membrane of the yeast *Saccharomyces cerevisiae* has a similar composition in ergosterol and also sphingolipids, although with smaller variety, to that of pathogenic fungi, being ergosterol the main classical target of antifungal agents [1]. In addition, *S. cerevisiae* sphingolipid-biosynthetic mutants, such as *ipt1*Δ, unable to synthesize the sphingolipid mannosyl-diinositolphosphorylceramide, $M(IP)_2C$, show greater antifungal resistance [2,3]. Both strains share equal content in ergosterol and therefore, the mechanisms of resistance may involve the highly-ordered sphingolipid-enriched domains discovered in our laboratory [4].

For the reasons above, it is important to study the sphingolipid influence in the plasma membrane organization of *S. cerevisiae*. To this end, we are isolating each complex sphingolipid class present in that organism to perform a biophysical characterization and compare it with biophysical properties observed in living cells. Before that it is, however, necessary that the sphingolipid extraction from yeast cells is optimized. After obtaining the total lipid extracts, the lipids have to undergo mild alkaline hydrolysis to eliminate glycerophospholipids. The sphingolipid extracts thus obtained are then analyzed by thin layer chromatography.

Regarding lipid extracts obtained by the Fölch method, it was possible to identify mannosyl-inositolphosphorylceramide (MIPC), in both *wt* and *ipt1*Δ extracts. The impossibility to detect $M(IP)_2C$ may be due to low extraction and/or revelation efficiency of this highly polar lipid and consequently additional methods were attempted. No differences in glycerophospholipids and ergosterol contents between the two strains were observed.

Preliminary biophysical studies with several sphingolipid biosynthetic mutants were performed in living cells, revealing sphingolipid-dependent changes of plasma membrane organization.

Acknowledgments: *Fundação para a Ciência e a Tecnologia (FCT), Portugal is acknowledged for PTDC/BBB-BQB/6071/2014, UID/Multi/00612/2013, and IF2012 initiative (POPH, Fundo Social Europeu).*

References

[1] Castelli, M. V. et al. Novel antifungal agents: a patent review (2011 - present). Expert Opin. Ther. Pat. 2014, 24, 323–338.

[2] François, I. E. J. A. et al. Membrane rafts are involved in intracellular miconazole accumulation in yeast cells. J. Biol. Chem. 2009, 284, 32680–32685.

[3] Leber, A., Fischer, P., Schneiter, R., Kohlwein, S. D. & Daum, G. The yeast mic2 mutant is defective in the formation of mannosyl-diinositolphosphorylceramide. FEBS Lett. 1997, 411, 211–214.

[4] Aresta-Branco, F. et al. Gel domains in the plasma membrane of Saccharomyces cerevisiae: highly ordered, ergosterol-free, and sphingolipid-enriched lipid rafts. J. Biol. Chem. 2011, 286, 5043–5054.

P10 - SUBTILASES: THE MISSING LINK OF GRAPEVINE RESISTANCE AGAINST MILDEW

Joana Figueiredo,[1,2,3] Gonçalo Costa,[4] Marisa Maia,[1,2,3] Clemente da Silva,[1] Octávio S. Paulo,[4] Rui Malhó,[1] Marta Sousa Silva[2,3,*] and Andreia Figueiredo[1,*]

(1) *Biosystems & Integrative Sciences Institute (BioISI), FCUL, Portugal;* ***(2)*** *Laboratório de FTICR e Espectrometria de Massa Estrutural, FCUL, Portugal;* ***(3)*** *Centro de Química e Bioquímica, FCUL, Portugal;* ***(4)*** *Computational Biology and Population Genomics Group, Centre for Ecology, Evolution and Environmental Changes (cE3c), FCUL, Portugal;*

(*) *co-senior authors*

fc41899@alunos.fc.ul.pt

Grapevine (*Vitis vinifera* L.), the most important fruit plant cultivated worldwide due to its economic importance in the wine industry, is highly susceptible to downy mildew, caused by *Plasmopara viticola*. Our studies in this pathosystem highlighted the role of a subtilase presenting a high constitutive expression in a resistant *V. vinifera* cultivar and a high increase in the first hours of *P. viticola* inoculation. Studies in other plants systems have highlighted subtilase participation in response to biotic and abiotic environment stimulus. In tomato leaves the increase of expression of a subtilase (P69) was observed after a viroid infection. Also, in *A. thaliana* a subtilase gene (SBT3.3) which expression rapidly increases during innate immunity activation was identified. We characterized the grapevine subtilase gene family and identified 97 proteins phylogenetically divided into 6 groups and predictably located in apoplast, cell wall or extracellular region. Of these, 14 subtilases presented either high homology to tomato P69C, *A. thaliana* SBT3.3 and are located near the *Resistance to Plasmopara viticola* locus. Expression studies in this pathosystem indicate that some of grapevine subtilases are actively participating in the defence response against *P. viticola* [1]. Two of them were cloned and the first tests for recombinant protein expression are underway. These results are the first step in the direction of the establishment of these subtilases as the missing link in grapevine resistance against downy mildew and future candidates for introgression in breeding programs, as an alternative to the excessive use of pesticides.

Acknowledgments: *This work was supported by projects PEst-OE/BIA/UI4046/2014, PEst-OE/QUI/UI0612/2013, UID/MULTI/00612/2013, and grant SFRH/BPD/99712/2014 from Fundação para a Ciência e Tecnologia (FCT/MCTES/PIDDAC, Portugal), and by the European FP7 project PERSSILAA (grant agreement 610359).*

References

[1] Figueiredo, J., Costa, G. J., Maia, M., Paulo, O. S., Malhó, R., Sousa Silva, M., & Figueiredo, A. (2016). Revisiting *Vitis vinifera* Subtilase Gene Family: A Possible Role in Grapevine Resistance against *Plasmopara viticola*. *Frontiers in Plant Science*, 7, 1783. http://doi.org/10.3389/fpls.2016.01783.

P11 - CFTR EXIT FROM THE ER: IDENTIFICATION OF NOVEL TRAFFIC FACTORS

João Delgado Santos,[1] Ana Sofia Carvalho,[2] Kerman Aloria,[3] Rune Matthiesen,[2] Manuela Zaccolo[4] and Carlos Miguel Farinha[1]

(1) *Functional Genomics and Proteostasis Group, Biosystems and Integrative Sciences Institute, Faculdade de Ciência, Universidade de Lisboa, Lisboa, Portugal;* ***(2)*** *Computational and Experimental Biology Group, CEDOC – Chronic Diseases Research Center, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal;* ***(3)*** *Faculdad de Ciencia y Tecnologia, Universidad del Pais Vasco UPV/EHU, Vizcaya, Spain;* ***(4)*** *Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom*

jfsantos@fc.ul.pt

Introduction: Cystic Fibrosis (CF) is an autosomal recessive genetic disorder caused by dysfunction of the CFTR protein [1]. The most common disease-causing mutation (F508del, present in 70% of CF chromosomes) leads to CFTR misfolding which is recognized by the endoplasmic reticulum (ER) quality control (ERQC) resulting in ER retention and early degradation [2]. The retention of misfolded CFTR at ER is mediated by the exposure of arginine-framed (RXR) tripeptides (AFTs) however, the recognition mechanism is not clear [3]. We aim to identify AFT interactor proteins involved in CFTR exit from the ER that may be used as novel therapeutic targets in the rescue of F508del-CFTR.

Methods: Pull-down assays using Cystic Fibrosis Bronchial Epithelial (CFBE) parental cells were performed to isolate AFT specific interactors. Synthetic peptides conjugated with agarose beads specifically designed were used to mimic mutated or non-mutated CFTR protein at the AFT regions. Samples were analyzed by LC-MS/MS method and proteins showing differential interactions with the two sets of peptides were selected. The DAVID database and GSEA computational tool were used to analyses AFT interactors.

Results and Discussion: A high number of AFT interactors were identified with the majority corresponding to proteins localized to the ER or involved in cytoskeleton regulation. Several of these interactors were not previously directly associated with CFTR regulation.

The identification of the specific CFTR interactors/regulators, and its validation which is in progress, is a promising approach in the identification of novel therapeutic targets that could be ultimately used to the benefit of CF patients.

Acknowledgments: *Work supported by: PEst-OE/BIA/UI4046/2011 to BioISI (from FCT – Portugal). João Delgado Santos PhD fellowship: PD/BD/106084/2015 (from FCT – Portugal). The authors wish to thank to Prof. Francisco Pinto for the help in the bioinformatic analysis.*

References

[1] Riordan JR. Ann Rev Biochem. 2008;77(1):701–26.

[2] Farinha CM, Amaral MD. Mol Cell Biol. 2005;25(12):5242–52.

[3] Farinha CM, Matos P, Amaral MD. FEBS J. 2013;280(18):4396–406.

P12 - ESTABLISHMENT OF A MODEL FOR ASSESSING CFTR AND KLF4 ROLES IN EPITHELIAL DIFFERENTIATION

Luis M. Sousa[1], Ines Pankonien[1], Marc Chanson[2], Margarida D. Amaral[1]

(1) *University of Lisboa, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Lisboa, Portugal;* ***(2)*** *Département de Physiologie Cellulaire & Métabolisme, Centre Médical Universitaire, Geneva, Switzerland*

lmdsousa@fc.ul.pt

The airway epithelium of Cystic Fibrosis (CF) patients is subject to constant damage and repair due to recurrent bacterial infections and inflammation [1]. Several studies have shown that wound healing and cell differentiation are both altered in CF [2,3]. Moreover, it has also been suggested that functional CFTR and epithelial differentiation are strongly associated [4,5].

The initial goal of this work is to establish cell-based assays of airway epithelial differentiation and wound healing to be used in automated microscopy siRNA screens. Our final goal is to validate the role of the obtained hits in epithelial differentiation and CFTR maturation in a CF vs. non-CF context. One of the first hits identified was Krüppel-like factor 4 (KLF4) which plays a role in differentiation and was thought to modulate CFTR traffic.

We have used Cystic Fibrosis Bronchial Epithelial (CFBE) cells stably overexpressing wt-CFTR or F508del-CFTR and observed that F508del-CFTR cells display slower wound healing. On the other hand, KLF4 knock-out cells displayed faster wound healing and enhanced wt-CFTR maturation and expression. Moreover, we have developed cells expressing proliferation and differentiation markers reporters to be used later in the characterization of the role of CFTR in wound healing, cell proliferation and differentiation.

Acknowledgments: *Work supported by FCT Grant "DiffTarget" FCT/POCTI (PTDC/BIM-MEC/2131/2014) and by PD/BD/106087/2015.*

References

[1] Strausbaugh SD, Davis PB. Cystic Fibrosis: A Review of Epidemiology and Pathobiology. Clinics in Chest Medicine. 2007;28(2):279-88.

[2] Trinh NT, Prive A, Maille E, Noel J, Brochiero E. EGF and K+ channel activity control normal and cystic fibrosis bronchial epithelia repair. American journal of physiology Lung cellular and molecular physiology. 2008;295(5):L866-80.

[3] Meyerholz DK, Stoltz DA, Namati E, Ramachandran S, Pezzulo AA, Smith AR, et al. Loss of Cystic Fibrosis Transmembrane Conductance Regulator Function Produces Abnormalities in Tracheal Development in Neonatal Pigs and Young Children. American journal of respiratory and critical care medicine. 2010;182(10):1251-61.

[4] Larson JE, Delcarpio JB, Farberman MM, Morrow SL, Cohen JC. CFTR modulates lung secretory cell proliferation and differentiation. American journal of physiology Lung cellular and molecular physiology. 2000;279(2):L333-41.

[5] Bebok Z, Venglarik CJ, Panczel Z, Jilling T, Kirk KL, Sorscher EJ. Activation of DeltaF508 CFTR in an epithelial monolayer. The American journal of physiology. 1998;275(2 Pt 1):C599-607.

P13 - STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE CATIONIC ANTIMICROBIAL PEPTIDE ECAMP1R2

Marcin Makowski,[1] Mário R. Felício,[1] Octávio L. Franco,[2,3] Nuno C. Santos[1] and Sónia Gonçalves[1]

(1) *Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal;* ***(2)*** *S-inova Biotech, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande-MS, Brazil;* ***(3)*** *Centro de Anláises Proteômicas e Bioquimicas/Programa de Pós Graduação em Ciências Genômicas e Biotecnologia, UCB, Brasília, DF, Brazil.*

marcin.makowski@medicina.ulisboa.pt

Tackling antimicrobial resistance is a worldwide priority. Antimicrobial peptides (AMPs) are among the most promising alternatives to conventional antibiotics. The main target of these molecules is the negatively charged membranes of bacteria, which, after interaction are eventually killed through diverse modes of action.

A recently discovered AMP, *Ec*AMP1R2, has showed antimicrobial activity against *Escherichia coli* at 11.7 μM. In order to shed some light on the mechanisms of action at the molecular level of this peptide, studies using biomembrane models that mimic bacterial composition and *E. coli* cells were assessed. Large unilamellar vesicles (LUVs) with different lipid compositions were used for this purpose, namely POPC/cholesterol (characteristic of the outer leaflet of mammalian cell membranes) and POPC/POPG (Gram negative bacteria) mixtures. Changes on membrane packing, fluidity and membrane potential were followed by the extrinsically fluorescence of molecular probes: Laurdan, TMA-DPH, DPH and di-8-ANEPPS, upon membrane binding/insertion. Both structural (based in *in silico* predictions and circular dichroism studies) and functional analysis (based in fluorescence and light scattering spectroscopy techniques) have been performed. *Ec*AMP1R2 displayed higher partition constant values (*Kp*) when interacting with negatively charged membranes (POPC:POPG). Interestingly, the studies using the fluorescent probe di-8-ANEPPS have revealed that the peptide produces a stronger depolarization in *E. coli* compared to the lipid mixtures analyzed. The results obtained suggest that the initial activity exerted by *Ec*AMP1R2 is highly dependent upon electrostatic interactions. Based on this, we suggest that the bacterial growth inhibition effect might be exerted by the disruption of the bacterial cell electric homeostasis.

P14 - INVESTIGATIONS OF S100 PROTEINS AGGREGATES AND ZINC LEVELS IN BRAINS OF ALZHEIMER'S DISEASE MICE MODELS

Mariana A. Romão,[1] Simone Hagmeyer,[2] Andreas M. Grabrucker[2] and Cláudio M. Gomes[1]

(1) *Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal and Departmento de Química e Bioquímica, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *WG Molecular Analysis of Synaptopathies, Neurology Department, Neurocenter of Ulm University, Ulm, Germany and Department of Biological Sciences, University of Limerick, Limerick, Ireland*

maromao@fc.ul.pt

A common feature in age-related neurodegenerative disorders is the occurrence of protein aggregation and activation of inflammatory pathways. Misfolding of normally soluble proteins results in their subsequent conversion into toxic amyloid aggregates which is accompanied by upregulation of pro-inflammatory cytokines such as S100 proteins. S100s are small (12kDa) Ca^{2+}-binding signaling proteins which occur mostly as homodimers. Ca^{2+} binding occurs at two EF-hand motifs and some homologues contain additional regulatory Zn^{2+}/Cu^{2+} binding sites.

S100 proteins are involved in numerous cellular pathophysiological processes and some neuronal S100s are consistently altered in neurodegeneration, including in Alzheimer's disease. Among these are S100A8, S100A9 and the heterodimer S100A8/A9 (calprotectin, CP) which seem to undergo self-assembly upon zinc and calcium binding,a process which is likely mechanistically linked to the cross-beta forming propensity which we have recently elucidated [1].

Here we report an investigation dealing with our hypothesis that zinc and calcium-binding to neuronal S100s promotes the formation of protein deposits in the AD brain and that this is related to changes in Zinc homeostatic levels, which are known to be altered in ageing and neurodegeneration. For the purpose, we analysed brain sections from APP23 AD mice models and different ages (3 and 15 months), using immunohistochemical analysis and fluorescence microscopy in combination with biochemical assays. We have analysed the occurrence of S100A8 and S100A9 protein assemblies in correlation to brain zinc levels and the presence of amyloid plaques. In this communication, we will report our preliminary results that pave the way to explore new roles for S100s as modifiers of Alzheimer's Disease.

Acknowledgments: *Support for this work was provided by grants from FCT/MCTES (PTDC/NEU-NMC/2138/2014 to CMG) and by Bial Foundation (343/14 to CMG). MAR acknowledges fellowship support from FCT/MCTES PD/BD/114390/2016 as a BioSYS PhD program student. This work is partly supported by the COST Action TD1304 The Network for the Biology of Zinc (Zinc-Net) via Short-Term Scientific Mission (STSM) travel grants (to M.A.R. and S.H.).*

References

[1] Carvalho, S. B., Botelho, H. M., Leal, S. S., Cardoso, I., Fritz, G., Gomes, C. M., Intrinsically Disordered and Aggregation Prone Regions Underlie β-Aggregation in S100 Proteins. *PLOS ONE*, **2013**, *8(10)*, e76629.

P15 - EXPLORING THE INTERACTIONS BETWEEN NEURON DEGENERATION AND RNA HOMEOSTASIS THROUGH BIOLOGICAL NETWORK ANALYSIS

Marina L. Garcia-Vaquero,[1] Margarida G. Carvalho,[1] Javier de Las Rivas[2] and Francisco R. Pinto[1]

(1) *BioISI: Biosystems and Integrative Sciences Institute. Faculdade de Ciências da Universidade de Lisboa, Campo Grande 1749-016 Lisbon, Portugal;* ***(2)*** *CIC: Cancer Research Center, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain.*

mlgarciavaquero@fc.ul.pt

Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) are characterized by Motor Neuron (MN) degeneration. MN-degeneration leads to the loss of muscle innervation and subsequent muscular atrophy. In addition to phenotypic similarity, they also share molecular overlaps. Genes that codify **FUS, TDP43** and **SETX** proteins are the best-known causative genes of ALS [1–4] while **SMN**-protein dysfunction is the cause of SMA [5] **(FTSS-proteins)**. Additionally, these proteins are known to share similar functions and physically interact [6]. This fact supports the hypothesis that ALS and SMA are different pathophenotypic results derived from related molecular origins.

In order to explore this question, we firstly performed an **interactomic and functional analysis** to unravel the most influential functions among FTSS proteins. Secondly, we developed **a new method, S2B (double specific betweenness)** to prioritize and identify proteins specifically linking a pair of diseases –novel DAG candidates-. Finally we functionally enriched the S2B-derived candidates and compared against the functional set obtained with FTTS-focused network in other to explore the putative molecular mechanisms involved in MN degeneration.

Globally, our results suggest **five pathways in common between ALS and SMA**: 1) DNA damage and apoptosis induced by R-loop deregulation, 2) neurodegeneration induced by immune hyper-sensitivity, 3) genotoxicity produced by histone biogenesis perturbation, 4) genotoxicity produced by spliceosome assembly failure and 5) deregulation of microtubule related processes leading to axon and synapse formation alteration.

Acknowledgments: *Work supported by grants from* ***1)*** *BioSys-PhD programme at BioISIS supported by grants through Fundação para a Ciência e Tecnologia (FCT), Portugal (PhD fellowship PD/BD/128109/2016) and* ***2)*** *FCT UID/MULTI/04046/2013 and JPND-CD/0002/2013. This is an EU Joint Programme- Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organizations under the aegis of JPND www.jpnd.eu (France/ANR; Germany/BMBF; Portugal/FCT; Spain/ ISCIII).*

References:

[1] Rutherford, N. J. *et al.* Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. *PLoS Genet.* 4, (**2008**);

[2] Sreedharan, J. *et al.* TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science (80-.).* 319, 1668–72 (**2008**);

[3] Kwiatkowski Jr, T. J. *et al.* Mutations in the FUS/TLS Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis. *Science (80-.).* 323, 1205–1209 (**2009**);

[4] Chen, Y. *et al.* DNA / RNA Helicase gene mutations in a form of Juvenile Amyotrophic Lateral Sclerosis (ALS4). *Am. J. Hum. Genet* 74, 1128–1135 (**2004**);

[5] Lefebvre, S. *et al.* Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80, 155–165 (**1995**); [6] Cooper, T. A., Wan, L. & Dreyfuss, G. RNA and Disease. *Cell* 136, 777–793 (**2009**).

P16 - "I HEARD IT THROUGH THE GRAPEVINE" THAT LEAVES ARE AN UPCOMING VALUABLE BY-PRODUCT

Marisa Maia,[1,2,3] Rui Nascimento,[2,3] António Ferreira,[1,2] Ana P. Marques,[1,2] Joana Figueiredo,[1,2,3] Ana Ponces,[2] Carlos Cordeiro,[1,2] Andreia Figueiredo[3]* and Marta Sousa Silva[1,2]*

(1) *Laboratório de FTICR e Espectrometria de Massa Estrutural, FCUL, Portugal;* ***(2)*** *Centro de Química e Bioquímica, FCUL, Portugal;* ***(3)*** *Biosystems & Integrative Sciences Institute (BioISI), FCUL, Portugal*

**co-senior authors*

fc40277@alunos.fc.ul.pt

Grapevine (*Vitis vinifera* L.) is the most important fruit crop in the world [1]. Nowadays, one of the main objectives of processing industries is related to waste treatment and valorisation, and the wine industry is no exception. The use of grapevine leaves, either for inclusion in the human diet or as a source of bioactive compounds, may become an important component in solving this task, as they provide a large reserve of antioxidants and other biologically active components. Hence, a complete understanding of their metabolic composition is extremely important, not only to understand their physiology and response under stress conditions, but also for productivity and quality improvement, metabolic pathway engineering and food safety assessment [2]. We developed a metabolite extraction method for grapevine leaves to increase metabolome coverage [3] and performed a large scale metabolic characterization of different *V. vinifera* cultivars using Fourier Transform Ion Cyclotron Resonance mass spectrometry (FTICR-MS). The identified metabolic entities were annotated and classified by database search into six major metabolic classes. With the increasing search for natural compounds, plant-based supplements and organic food products, grapevine leaves are a potential healthy alternative due to their composition in phenols, mainly flavonols with high antioxidant capacity, and anthocyanins. We also detected several drugs and pesticides in the different grapevine cultivars' leaves, highlighting the importance of having accurate detection methods for these chemicals in food products.

Acknowledgments: *The work presented in this article was part of M. Maia master thesis (2016). This work was supported by projects EXPL/BBB-BIO/0439/2013, REDE/1501/REM/2005, UID/MULTI/00612/2013, PEst-OE/QUI/UI0612/2013, PEst-OE/BIA/UI4046/2014 and grant SFRH/BPD/99712/2014 from Fundação para a Ciência e Tecnologia (Portugal), and by the European FP7 project PERSSILAA (grant agreement 610359).*

References

[1] Organisation of Vine and Wine, "2016 OIV Statistical Report on World Vitiviniculture," 2016.

[2] R. U., W. L., and F. A., "Metabolic profiling and biochemical phenotyping of plant systems," *Plant Cell Rep.*, vol. 21, no. 3, pp. 189–196, Oct. 2002.

[3] M. Maia *et al.*, "Metabolite extraction for high-throughput FTICR-MS-based metabolomics of grapevine leaves," *EuPA Open Proteomics*, vol. 12, pp. 4–9, Sep. 2016.

P17 - sncRNA REGULATORY NETWORKS IN T CELL ACTIVATION AND VIRAL RESPONSE

Nuno Domingues,[1] Francisco Pinto[1] and Margarida Gama-Carvalho[1]

(1) *University of Lisbon, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute, Campo Grande, 1749-016 Lisboa, Portugal*

nmcdomingues@fc.ul.pt

miRNAs act post-transcriptionally to inhibit the expression of specific messenger RNAs (mRNAs) and they have been described as critical modulators of adaptive immune responses [1]. Validating a specific miRNA function is still a daunting task due to the complexity of the interactions established by miRNA networks. Development of approaches based on the use of theoretical models that predict the functional impact of miRNAs on a given system would be of undeniable value. miR-34c-5p has been recently identified by the host laboratory as being induced in response to TCR stimulation of naive CD4+ T cells, but not in memory cells, suggesting a role in differentiation and memory formation [2]. Additionally, this induction was found to be blocked in response to HIV infection [2]. These results provide the first evidence that this microRNA plays an important role in immune system function, most likely by regulating the process of naive T cell activation and differentiation, which is also critical for HIV infection. However, the specific function of this miR and its functional impact still remains to be elucidated. In parallel, the available results regarding HIV infection suggest that the virus may have the ability to subvert T cell processes through virally encoded miRNA-like molecules. This projects aims to explore how miRNAs act to regulate CD4+ T cell function during activation and HIV infection, with a particular focus on miR-34c-5p and the identified HIV-encoded miR candidates using a combination of logical modelling of signaling and gene regulatory networks with miRNA regulation and experimental approaches to validate model predictions.

Acknowledgments: *The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) for the PhD scholarship PD/BD/106088/2015. Work supported by grants from FCT UID/MULTI/04046/2013.*

References

[1] Podshivalova, K. & Salomon, D.R., (2013) MicroRNA regulation of T-lymphocyte immunity: modulation of molecular networks responsible for T-cell activation, differentiation, and development. *Critical reviews in immunology*, 33(5).

[2] Amaral, AJ et al. (2016), miRNA profiling of human naive CD4 T cells links miR-34c-5p to cell activation and HIV replication. The EMBO Journal.

P18 - CHEMICAL CHARACTERIZATION OF *CYNARA CARDUNCULUS* VAR. *SCOLYMUS* AND ITS APPLICATION IN TOPICAL FORMULATIONS

P. Marques,[1] L. Gonçalves L,[2] J. Marto,[2] R. Pacheco,[3,4] H. Ribeiro[2] and M.L. Serralheiro[4,5]

(1) *Faculty of Sciences, University of Lisbon, Lisbon, Portugal;* ***(2)*** *Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, 1649-003 Lisbon, Portugal;* ***(3)*** *Area Departamental de Engenharia Química, ISEL, 1949-014 Lisbon, Portugal;* ***(4)*** *Centro de Química e Bioquímica of Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal;* ***(5)*** *Department of Chemistry and Biochemistry of Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal*

patriciaahmarques@gmail.com

Cynara cardunculus var. *scolymus*, usually known as artichoke, is a rich plant in polyphenols, including caffeoylquinic and dicaffeoylquinic acids, with therapeutic properties, such as antioxidant activity [1]. The use of bioactive ingredients extracted from plant tissues in cosmetics is increasing, thus artichoke extract due to its constituents can be incorporated in topical formulations. The aim of this study was to investigate the antioxidant potential of artichoke in keranocytes and its potential to be a valuable ingredient for cosmetic products with anti-aging and antioxidant properties. An infusion was performed to extract the bioactive ingredients of artichoke leaves and two purification methods were applied to improve its antioxidant activity. Aqueous extracts were analyzed by RP-HPLC-DAD to quantify target compounds, like cynarin, chlorogenic acid and cynaroside. Antioxidant activity by DPPH assay and ROS scavenging activity in HaCaT cells, as well as cytotoxicity and sun protection factor (SPF) assays, were assessed. The results showed that artichoke extract and one purify fraction were rich in polyphenols. Both fractions were incorporated in topical formulations: O/W emulsion and hydrogel. Physicochemical characterization, microbiological control and cytotoxicity assays were performed to ensure the quality, safety and efficacy of the products developed. *In vivo* studies, Human Repeat Insult Patch Testing (HRIPT) and an antioxidant activity assay, were also performed. Besides the excellent antioxidant and photoprotective activity, the final formulations proved to be also suitable for topical use according to the rheological assessment. Artichoke extract appeared to be a promising and efficient antioxidant with photoprotective properties when applied topically.

Acknowledgments: *The authors thank Fundação para a Ciência e Tecnologia for financial support: PEst-OE/QUI/UI0612/2013.*

References

[1] P. L. Falé, C. Ferreira, A. M. Rodrigues, P. Cleto, P. J. A. Madeira, M. H. Florêncio, F. N. Frazão, and M. L. M. Serralheiro, “Antioxidant and anti-acetylcholinesterase activity of commercially available medicinal infusions after in vitro gastrointestinal digestion” Journal of Medicinal Plants Research, vol. 7, no. 20, pp. 1370–1378, May 2013.

P19 - TRANSCRIPTOMIC SCREEN FOR DIS3, DIS3L1 AND DIS3L2-ASSOCIATED FUNCTIONAL NETWORKS IN COLORECTAL CANCER

Paulo JP da Costa,[1] Hugo Santos,[1] Margarida Gama-Carvalho[1,2] and Luísa Romão[1,3]

(1) *Biosystems and Integrative Sciences Institute (BioISI). Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon;* ***(3)*** *Instituto Nacional de Saúde Doutor Ricardo Jorge. Avenida Padre Cruz, 1649-016 Lisboa, Portugal*

paulo.gomes@insa.min-saude.pt

The final step of eukaryotic mRNA degradation proceeds in either a 5'-3' direction, catalyzed by XRN1, or in a 3'-5' direction catalyzed by DIS3, DIS3L1 (the catalytic subunits of the exosome) and/or DIS3L2 (exosome-independent). Important findings over the last years have shed a new light onto the mechanistic details of RNA degradation by these exoribonucleases. In addition, it has been shown that they are involved in growth, mitotic control and important human diseases, including cancer. In this work, we aim to analyze how DIS3, DIS3L1 and DIS3L2 regulate the human transcriptome, and how their functional interactions modulate the transcriptional reprogramming of colorectal cancer cells. Each one of these nucleases was depleted by RNA interference in HeLa cells and levels of several endogenous targets was monitored by RT-qPCR. Our results show that these exoribonucleases are target specific and not directly involved in any known mRNA decay mechanisms such as nonsense-mediated mRNA decay (NMD). However, we do not know yet what defines such target preference.

In parallel, our bioinformatics analysis of available transcriptomic data from cells depleted of DIS3L1, DIS3L2, XRN1 or UPF1 (which has a central role in NMD) has shown some, but not full, redundancy among the transcripts regulated by these nucleases, which supports our experimental data.

Presently, we are exploring the mechanism through which DIS3L2 is involved in NMD and how it modulates the expression of NMD targets.

Acknowledgments: *Paulo JP da Costa acknowledges financial support from the FCT foundation (PD/BD/52495/2014). This work was partially supported by Fundacão para a Ciência e a Tecnologia (PTDC/BIM-MEC/3749/2014 and UID/MULTI/04046/2013 to BioISI from FCT/MCTES/PIDDAC).*

P20 - THE IMPORTANCE OF UNSTRUCTURED TERMINI IN THE AGGREGATION CASCADE OF BETA-2-MICROGLOBULIN: INSIGHTS FROM MOLECULAR SIMULATIONS OF D76N MUTANT

Rui João Loureiro,[1] D.V. Viçosa, [2] M. Machuqueiro[2] and Patrícia F.N. Faísca[1,3]

(1) *BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa;* ***(2)*** *Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa;* ***(3)*** *Departamento de Física, Faculdade de Ciências da Universidade de Lisboa*

rjloureiro@fc.ul.pt

The identification of intermediate states for folding and aggregation is important, both from a fundamental standpoint and for the design of new therapies targeted at conformational disorders. Here, we use the single point mutant (D76N) of β2m, the causing agent of a hereditary systemic amyloidosis affecting visceral organs, as a model system to study the aggregation mechanism of β2m using molecular simulations. We present our predictions on the early molecular events triggering the amyloid cascade for the D76N mutant. Folding simulations highlight the existence of an aggregation-prone intermediate called I1 which presents an unstructured C-terminus and of an aggregation-prone intermediate featuring two unstructured termini called I2. Additionally, Monte Carlo docking simulations suggest that the I2 is considerably more amyloidogenic than I1. These simulations support an essential role of the DE-loop and of the C-terminus in the dimerization of both intermediates. The relevance of the C-terminus is higher at the acidic pHs 5.2 and 6.2. Additionally, the AB-loop becomes an important player in the dimerization of both I1 and I2 at pH 6.2. A key specific finding of I2 dimerization is the relevance of Tyr 10 at the end of strand A at pH 6.2. These predictions rationalize experimental results that support the involvement of the AB-loop and DE-loop, particularly of Glu 16, Lys 19, Phe 56, Trp 60 and Tyr 63, in amyloidogenesis in the wild-type and other model systems of β2m.

Acknowledgments: *RJL thanks FCT for financial support through doctoral fellowship PD/BD/108259/2015. PFNF and RJL thanks FCT for financial support through grant UID/MULTI/04046/2013 from FCT/MCTES/PIDDAC (to BioISI).*

P21 - THE ROLE OF CODON USAGE IN THE EXPRESSION AND FOLDING OF THE DISEASE-RELATED METABOLIC ENZYME ETF:QO

Tânia G. Lucas,[1] Ana Tenreiro,[2] Bárbara J. Henriques,[1] João V. Rodrigues[3] and Cláudio M. Gomes[1]

(1) *Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal, and Departmento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal and Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(3)*** *Current address: Harvard University, Boston, USA*

tglucas@fc.ul.pt

Electron transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO) is a structurally complex 64 kDa protein which binds to the inner mitochondrial membrane, and contains three different cofactors ([4Fe-4S], FAD, ubiquinone) organized in distinct structural domains [1,2]. ETF:QO, together with its partner ETF, is an important hub in mitochondrial metabolism. Defects in these two proteins result in multiple acyl-CoA dehydrogenase deficiency (MADD), a rare metabolic disease [1,3,4]. We seek to elucidate the molecular and structural origins of the broad palette of MADD phenotypes, a hard task due to the difficulty to express and purify this complex membrane protein. Towards this goal, we here report a *tour de force* approach that involved to generate and express in *E. coli* 48 different ETF:QO constructs, corresponding to a combination of protein truncated and tagged variants. Using this strategy we achieved successful expression and purification of active and folded ETF:QO, which was a major progress in the field as the recombinant human ETF:QO had never been successfully expressed in a bacterial system. We then investigated the possible origins for the previous difficulty in protein expression, and we analyzed for possible codon-usage effects, a factor known to influence and regulate protein folding [5]. Using fluorophores specific for misfolded proteins coupled to cell sorting and biochemical and spectroscopic protein analysis we observed that constructs designed with optimized codon for bacterial expression resulted in higher expression level, albeit at expenses of a compromise in protein quality, with effects on decreased protein solubility, cofactor incorporation, and defective protein folding and function. Our results thus suggest that synonymous codons variants are effective modulators of translation rate and co-translational protein folding mechanisms.

Acknowledgments: *This work was supported by the Fundação para a Ciência e Tecnologia (FCT/MCTES, Portugal) through fellowships to T.G.L. (SFRH/BD/89690/2012) and B.J.H. (SFRH/BPD/74475/2010) and a grant PTDC/BBBBQB/5366/2014 (to B.J.H.). The Gomes laboratory is partly supported by grant UID/MULTI/04046/2013 from FCT/MCTES/PIDDAC (to BioISI). The P-cube consortium at Oxford University is acknowledged for construct generation.*

References

[1] Goodman, S.I., et al., *Mol Genet Metab.* **2002**, 77(1-2), 86-90.

[2] Wen, B., et al., *Mol Genet Metab.* **2013**, 109(2), 154-60.

[3] Henriques, B.J., et al., *J Biol Chem.* **2009**, 284(7),4222-9.

[4] Watmough, N.J. and F.E. Frerman, *Biochim Biophys Acta.* **2010**, 1797(12), 1910-6.

[5] Chaney, J. L., Clark, P. L., *Annu. Rev. Biophys*. **2015**, 44, 143-66 .

P22 - A HIGH-THROUGHPUT SCREENING ASSAY TO IDENTIFY FACTORS CORRECTING CFTR MUTATIONS BEARING PREMATURE TERMINATION CODONS (PTCS)

Verónica Felício,[1] Hugo M. Botelho,[1] Susana Igreja[1] and Margarida D. Amaral[1]

(1) *University of Lisboa, Faculty of Sciences, BioISI-Biosystems and Integrative Sciences Institute, Lisboa, Portugal.*

vmfelicio@fc.ul.pt

Background: Nonsense mutations, which introduce premature stop codons account for about 8% of the ~2,000 CFTR gene variants reported at CFTR Mutation Database [1]. These mutations usually lead to extensive transcript degradation by nonsense-mediated mRNA decay (NMD) thus preventing protein production. Such mutations are therefore associated with severe CF phenotypes. There is thus an unmet need to treat patients with these mutations and novel therapeutic strategies should be explored.

Objective: Our aim here is to identify novel factors that correct the defective processing of these transcripts and to validate them as novel drug targets.

Methods: To achieve this goal we are using a fluorescent CFTR mini-gene model harbouring the G542X mutation, which not only leads to the production of significantly lower levels of CFTR transcripts but also recapitulates the full NMD process. The NMD correction was assessed by: (i) semi-quantitative RT-PCR; (ii) high-throughput (HT) microscopy analysis using a ratiometric readout (eGFP/mCherry).

Results and Discussion: Our RT-PCR data show that levels of G542X-CFTR transcripts in non-treated and SMG-1 inhibitor treated cells were: 20% and 60%, respectively. By fluorescence microscopy we could not determine presence of eGFP fluorescence in SMG1-treated cells because SMG-1 inhibition only prevents NMD but has no readthrough activity. However, we observed a 5-fold increase in mCherry fluorescence. We are currently using this CFTR-NMD reporter to identify novel NMD factors by screening a previously validated shRNA library which is enriched in shRNAs targeting genes known or predicted to be involved in transcript processing (425 genes), using HT microscopy.

Acknowledgments: *Work supported by UID/MULTI/04046/2013 centre grant from FCT, Portugal (to BioISI) and CFF grant Ref. AMARAL15XX1 "RNA Life" (to MDA). VF is recipient of PhD fellowship (SFRH/BD/87478/2012) and SI and HMB by Post-doctoral fellowships (SFRH/BPD/85363/2012 and SFRH/BPD/93017/2013, respectively), all from FCT, Portugal.*

References

[1] http://www.genet.sickkids.on.ca/StatisticsPage.html.

P23 - NEW IRON(II) AND COPPER(II) COMPLEXES WITH CYCLAMS: SYNTHESIS AND APPLICATION IN CANCER THERAPY

Adhan Pilon,[1,2] M. Helena Garcia,[1] Fernanda Marques,[3] Ana M. Martins,[2] Andreia Valente,[1] Luís G. Alves[2]

(1) *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Centro de Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal;* ***(3)*** *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, E.N.10, 2695-066 Bobadela LRS, Portugal.*

adhanpilon@hotmail.com

Cancer is one of the main causes of death worldwide, being imperative continuous research in this field. Up to date, cisplatin (*cis*-$[Pt(Cl)_2(NH_3)_2]$) has been one of the mostly used drug to treat this disease worldwide [1]. Other metallodrugs have been revealing important cytotoxic properties, fueling the research in this area.

The work here described is placed within this subject through the functionalization of organic molecules with known biological activity, cyclams [2], with iron and copper centers envisaging a synergic biological effect. Thus, the synthesis and characterization of new cyclams and new Fe(II) and Cu(II) compounds is presented (**Figure 1**).

The cytotoxic potential of the new compounds was evaluated in the human breast adenocarcinoma cell line (MDA-MB-231) that is known to be highly aggressive and without cure yet. All this compounds present cytotoxicity in the micromolar range, similar to those observed for cisplatin in the same experimental conditions. The copper(II) complex was the one presenting the best cytotoxicity, twice better than cisplatin.

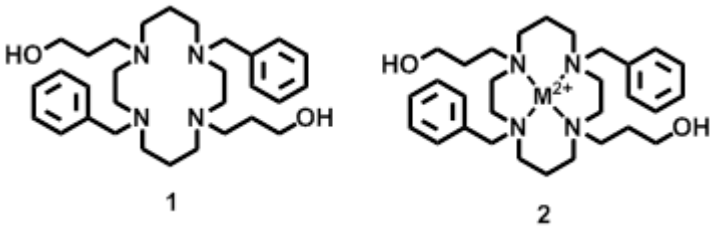


Figure 1- Cyclam structure (**1**) and general structure of the coordination compounds (**2**).

Acknowledgments: *This work was financed by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia, FCT) within the scope of project SFRH/BPD/86815/2012 and UID/QUI/00100/2013. Andreia Valente acknowledges the Investigator FCT2013 Initiative for the project IF/01302/2013 (acknowledging FCT, as well as POPH and FSE - European Social Fund).*

References

[1] Gasser G, Ott I, Metzler-Nolte N.; Organometalic Anticancer compounds; J. Med. Chem.; 2011, 54:3-25.

[2] Mingfeng Y, James A. T.; Nontoxic Metal−Cyclam Complexes, a New Class of Compounds with Potency against Drug-Resistant Mycobacterium tuberculosis; J. Med. Chem. 2016, 59, 5917−5921.

P24 - SYNTHESIS OF NEW RUTHENIUM COMPLEXES FOR THE TREATMENT OF PITUITARY ADENOMAS AND GLIOMA

Anabela Sanches,[1] Fernanda Marques,[2] M. Helena Garcia[1] and Andreia Valente[1]

(1) *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, E.N.10, 2695-066 Bobadela LRS, Portugal.*

anabela.fernandes.sanches@hotmail.com

The pituitary gland regulates many functions of other endocrine glands and their target tissues throughout the body. Consequently, every organ or tissue are affected, directly or indirectly, when occurs disorders in pituitary gland. Pituitary adenomas do not have a well-defined cause, which has aroused a special interest to this benign tumor, since it has a considerable impact on human well-being. [1]

During the last few years our research group has developed organometallic ruthenium(II)-cyclopentadienyl complexes that showed important cytotoxicity against several cancer cell lines, [2] which potentially can be also used to treat benign tumors. In this context, we synthetized and characterized new ruthenium(II)-cyclopentadienyl based complexes, with general the formula $\{Ru(\eta^5\text{-}C_5H_5)[P(C_6H_5)C_6H_4COOR]_nL\}^+$ (n = 2, R = H, L = Cl **1**; n = 1, L = 2-benzoylpyridine and R = H **2** or R = polylactide-OC_7H_7 **3**). The cytotoxic activity of the complexes was evaluated in two non-tumor cell lines of pituitary adenoma, GH3, secreting prolactin and growth hormone, and MMQ secreting prolactin. The cytotoxic potential against the highly aggressive human glioma tumor cell line U87 was also evaluated. The compounds did not present relevant cytotoxic activity in the pituitary adenoma cell lines studied, when the study is carried out in the absence of light. These first results are very promising for photodynamic therapy, since the compounds are expected to be cytotoxic only under irradiation. The intense metal ligand charge transfer (MLCT) band in the visible light revealed by the electronic spectra of these compounds encourages the study of their cytotoxicity under irradiation at MLCT energy. The present communication reports the first organometallic family of compounds designed to envisage the search of potential drugs to treat pituitary adenoma.

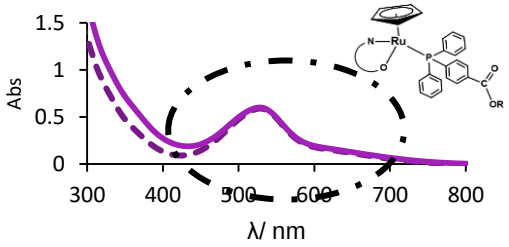


Figure 1 – Electronic spectrum of complexes (2) and (3) in dimetylsulfoxide.

Acknowledgments: *This work was financed by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia, FCT) within the scope of project UID/QUI/00100/2013. Andreia Valente acknowledges the COST action CM1302 (SIPs), the Investigator FCT2013 Initiative for the project IF/01302/2013 (acknowledging FCT, as well as POPH and FSE - European Social Fund).*

References

[1] Asa S. L., Ezzat S., The Annual Review of Pathology, **2009**, 4, 97-126; [2] T. S. Morais, A. Valente, A. I. Tomaz, F. Marques, M. Helena Garcia, Future Med.Chem.**2016**, 8(5), 527-544.

P25 - TITANATE NANOTUBES AND NANOWIRES MODIFIED WITH ETHYLENEDIAMINE FOR PHOTOCATALYTIC DEGRADATION OF PSYCHOACTIVE SUBSTANCES

B. Barrocas,[1] M.C. Neves[2] and O.C. Monteiro[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *CICECO, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.*

btbarrocas@fc.ul.pt

Nowadays many thousands of pharmaceuticals and personal care products (PPCPs) are used worldwide, and, after use, they are disposed in the environment. Several removal methodologies have been proposed but clear improvements in effectiveness and efficiency are needed. In this work, new hybrid nanomaterials, with improved photocatalytic performance for emergent pollutants removal, were obtained through sensitization of titanate nanotubes (TNT) and nanowires (TNW) with ethylenediamine (EDAmine) to produce NTNT and NTNW materials, respectively. The prepared materials were structural, morphological and optical characterized by XRD, TEM, DRS and XPS. The results show that TNT and TNW with identical diameter/length ratio, but distinct surface area, were obtained using the same experimental conditions (solvents, time and temperature) but different precursors [1-3]. No modifications on the structure and morphology were detected after EDAmine incorporation but an increase on the visible light absorption and on the point of zero charge were observed.

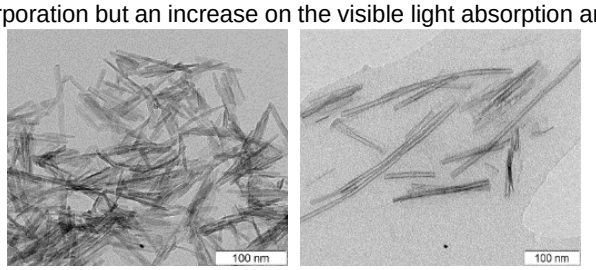


Figure 1 – TEM images of the TNW (a) and TNT (b) samples.

The application of these new hybrid nanomaterials on photocatalytic degradation of emergent pollutants was investigated. First, the evaluation of hydroxyl radical (•OH) production, using the terephthalic acid as probe was studied and the highest catalytic activity was achieved by the NTNT sample [4-6]. The photocatalytic ability of the sensitized materials for the psychoactive substances, caffeine and theophylline, and for phenol degradation was afterwards evaluated [4-6]. The results show that, within 60 min under UV-vis radiation, the NTNT sample was the best catalyst for all the degradation processes, achieving 60% of photodegradation efficiency for caffeine and 98% for phenol and theophylline 20 ppm solutions, respectively. Based on the obtained results, a mechanism for the charge-transfer processes in the NTNT hybrid nanoparticles, after being activated by the light, is proposed and discussed [4-6].

Acknowledgments: *The authors thank Fundação para a Ciência e Tecnologia for financial support (UID/MULTI/00612/2013, SFRH/BD/101220/2014 and IF/01210/2014).*

References

[1] M.R. Nunes, O.C. Monteiro, A.L. Castro, D.A. Vasconcelos, A.J. Silvestre, *Eur. J. Inorg. Chem.* **2008**, 28, 961; [2] V. Bem, M.C. Neves, M.R. Nunes, A.J. Silvestre, O.C. Monteiro, *J. Photochem. Photobiol. A* **2012**, 232, 50–56; [3] E.K. Ylhäinen, M.R. Nunes, A. J. Silvestre, O.C. Monteiro, *J. Mater. Sci.* **2012,** 47, 4305–4312; [4] B. Barrocas, A.J. Silvestre, A.G. Rolo, O.C. Monteiro, *Phys. Chem. Chem. Phys.* **2016,** 18, 18081-18093; [5] B. Barrocas, C.D. Nunes, M.L. Carvalho, O.C. Monteiro, *Appl. Surf. Sci.* **2016,** 385, 18–27; [6] T.A. Silva, J. Diniz, L. Paixão, B. Vieira, B. Barrocas, C.D. Nunes, O.C. Monteiro, *Solid State Sci.* **2017**, 63, 30–41.

P26 - EVALUATION OF ORGANIC MATERIALS OBTAINED FROM RENEWABLE SOURCES AS GREEN INHIBITORS OF CORROSION

Carla S. Proença,[1] Jorge Correia[1] and Maria Eduarda Araújo[1]

(1) *Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa. Campo Grande, 1749-016 Lisboa, Portugal*

csproenca@fc.ul.pt

Corrosion is a major problem that affects many metals and alloys. The costs and consequences caused by corrosion are of such importance that justifies a comprehensive study in relation to its causes, mechanisms of identification, detection and development of methods of prevention and protection. Among the technical materials, aluminum alloys are of upmost importance due to their broad application spectrum, from everyday objects like cooking pots, buildings and even in aircraft industry [1].

The former conventional pre-treatment of aluminum alloys involves formulations containing chromium (VI) but REACH (Registration, Evolution, Authorization and Restriction of CHemicals) restricts the use of hexavalent chromium in EU, due to the negative impact of these compounds in environment and human health. In the last few years there has been an increase in the interest in studying new corrosion inhibitors that could be efficient and more environmentally and human-friendly, to replace the chromium (VI) based treatments [1-3].

In this communication, we present the preliminary studies concerning the use of some organic compounds, obtained from renewable sources, as inhibitors in aluminum corrosion. The stability of solutions of such green inhibitors in sulfuric acid medium (0,46M) was monitored over time by UV-Vis spectroscopy during 12 months. The corrosion resistance of test samples of the alloy AA2024-T3 treated by 60 min immersion was evaluated by electrochemical assays in aerated brine solutions. The results show that these compounds can be explored in corrosion prevention of aluminum alloys.

Acknowledgments: *This work is funded by FCT (Fundação para a Ciência e a Tecnologia) through the project UID/MULTI/00612/2013.*

References

[1] Capelossi V.R. et al, Electrochimic Acta, **2014,** 124, 69-79.

[2] Domingues L., Fernandes J.C.S., da Cunha Belo M., Ferreira M.G.S., Guerra-Rosa L., Corrosion Science, **2003**, 45, 149-160.

[3]Umoren S.A., M.M. Solomon, The Open Materials Science Journal, 2014, 8, 39-54.

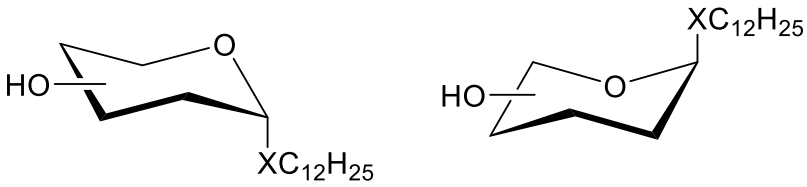
P27 - STRUCTURAL OPTIMIZATION OF ALKYL DEOXY GLYCOSIDES AS INNOVATIVE ANTIBACTERIAL AGENTS

C. Dias,[1] J. P. Pais,[1] R. Nunes,[1] A. Pelerito,[2] R. F. M. De Almeida[1] and A. P. Rauter[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *Instituto Nacional de Saúde Doutor Ricardo Jorge, 1749-016 Lisboa, Portugal;*

cadias@fc.ul.pt

The search for new antimicrobial drugs is currently one of the major ongoing research areas due to the spread of multidrug-resistance, making research on new antibacterial agents with new mechanisms of action highly relevant. The low toxicity, bioavailability and sustainability of sugar-based surfactants make them a very appealing class of compounds, with several applications in industry [1,2]. Indeed, alkyl 2-deoxy/2,6-dideoxy-*arabino*-hexopyranosides with a potent antimicrobial activity against *Bacillus* species have been previously described by our research group.[2] These results motivated us to synthesize new alkyl 2-deoxyglycosides to investigate the effect of small structural and configurational changes, envisioning the recognition of the structural features that determine bioactivity against *B. anthracis*. The importance of the configuration of hydroxy groups of the sugar moiety was assessed, along with the replacement of the exocyclic oxygen by sulphur in the glycosidic linkage. The deoxygenation pattern was also analysed and synthetic methodologies towards new alkyl 3-deoxy, 4-deoxy and 6-deoxy glycosides were investigated. The antimicrobial activity of these newly synthesized compounds was studied and will also be presented. The action of the lead compound on the thermotropic behaviour of phosphatidylethanolamine liposomes was investigated using biophysical techniques such as anisotropy fluorescence, leading to the proposal of the mechanism of action of such molecules. These findings, along with antimicrobial results, will be discussed and demonstrated the uniqueness of carbohydrates as exceptional scaffolds for medicinal chemistry applications.



Scheme 1. Structure-type of the antibacterial compounds. (X=O or S).

Acknowledgments: *This work was supported by FEDER-QREN-SI I&DT (Project n. 21457). The authors thank the FCT for financial support (UID/MULTI/00612/2013), and FCT and Cipan for the Ph.D. grant of C. Dias (SFRH/BDE/51998/2012).*

References

[1] A. Martins, M. S. Santos, C. Dias, A. P. Rauter *et al*. *Eur. J. Org. Chem.* **2013**, *8*, 1448-1459.

[2] A. P. Rauter, S. Lucas, T. Almeida, D. Sacoto *et al*. *Carbohydr. Res*. **2005,** *340*, 191-201

[3] F. V. M. Silva, M. Goulart, A. P. Rauter *et al*. *Bioorg. Med. Chem*. **2008,** 16, 4083-4092.

P28 - EVIDENCE OF A NEW POLYMORPH IN 4'-HYDROXYVALEROPHENONE AND 4'-HYDROXYHEPTANOPHENONE

Cátia S.D: Lopes,[1] Carlos, E.S. Bernardes, [1] M. Fátima M. Piedade, [1, 2] Hermínio P. Diogo[2] and Manuel E. Minas da Piedade[1]

(1) *Centro de Química e Bioquímica e Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *Centro de Química Estrutural, Complexo Interdisciplinar, Instituto Superior Técnico, 1049-001, Lisboa, Portugal.*

csolopes@fc.ul.pt

Molecular organic compounds can often exist in different crystal forms. This phenomenon is called polymorphism. Each polymorph can be considered a different material, since it shows dissimilar physical properties (e.g. fusion point, solubility). Hence, a reasonable understanding of this phenomenon is extremely important, to control the crystal phase (polymorph), morphology, and size distribution of the material. One approach to study this process is to investigate how intermolecular interactions (e.g. hydrogen bonds, Van der Waals) affect the crystal packing. Within this scope 4'-hydroxybenzoil (HOC_6H_4COR, R = H, *n*-alkyl) compounds constitute a model system with both hydrogen bond donor and acceptor groups connected to a benzene ring, and different size alkyl chains, that allow the modification of the Van der Waals interactions in the system. Previously, two different polymorphs for 4-hydroxybenzaldehyde (R = H) and 4'-hydroxyacetophenone (R = CH_3) were identified. This fact suggests these compounds are prone to polymorphism. Hence, in this work, a systematic study was performed to understand how growing of the alkyl chain may affect the packing architecture and the tendency to produce new polymorphs. This research lead to the discovery of two new phases for 4'-hydroxyvalerophenone (HVP, R = C_4H_9) and 4'-hydroxyheptanophenone (HHP, R = C_6H_{13}). These new polymorphs were characterized by differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and single crystal X-ray diffraction (SCXRD), enabling the discussion of their relative stability.

Acknowledgments: *This work was supported by Fundação para a Ciência e a Tecnologia, Portugal (Project UID/MULTI/00612/2013 and UID/QUI/00100/2013).*

P29 - TRIPODAL AMINOPHENOLATE METAL COMPLEXES WITH POTENTIAL THRAPEUTICAL ACTIVITY

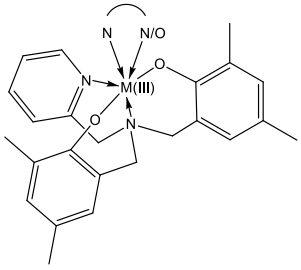
Cristina P. Matos,[1,2] M. Helena Garcia,[2] João Costa Pessoa,[1] Ana Isabel Tomaz[2] and Isabel Correia[1]

(1) *Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal;* ***(2)*** *Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

cristina.matos@tecnico.ulisboa.pt

Cancer remains one of the most challenging diseases and cancer-related deaths are estimated to rise as life expectancy increases. The severe toxic side-effects of platinum drugs, albeit their efficacy, led to a search for alternatives. Ruthenium-based compounds are recognized as promising anti-cancer metallodrugs with improved pharmacological properties and different mechanisms of action. An emerging approach in this field, aiming to regulate the cytotoxic responses of metallodrugs, is to use biologically essential transition metals. Iron, being redox-active and involved in the regulation of cell-growth and differentiation, has emerged as an appealing candidate.

Iron(III)-complexes containing phenolate ligands with tripodal amines attracted interest as mimics of enzyme active sites and metal-binding sites of iron proteins, but few reports describe their application as therapeutic agents. [1] Tripodal aminophenolate compounds are quite versatile ligands, as substituents at the phenolate rings, as well as the position and nature of the donor atoms are easily tunable features. [2] Since iron and ruthenium are isostructural, the development of tripodal aminophenolate complexes with both metal ions allows the comparison of the metal ion effect. The introduction of a NN/NO aromatic heterocyclic co-ligand in these complexes could enforce their biological activity, as metal complexes containing, for example, phenanthrolines, are reported to be active against various pathologic conditions. [3] A wide array of electronic features and redox potentials are thus available, which can direct synthetic strategies towards attaining stable complexes with optimal performance. This is the approach we have been using to develop new robust ruthenium/iron complexes with high potential for therapeutic applications.



Acknowledgments: *Authors thank Fundação para a Ciência e Tecnologia (FCT), the Investigador FCT programme 2012 and 2013 (IF/01179/2013, POPH and the European Social Fund), the Portuguese NMR Network (IST Nodes), UID/QUI/00100/2013 and the PhD research grant SFRH/BD/101214/2014.*

References

[1] M. Palaniandavar, M. Velusamy, R. Mayilmurugan, *J. Chem. Sci.* **2006**, *118*, 601-610.

[2] T. Marshall-Roth, S. N. Brown, *Dalton Trans*. **2015**, *44*, 677-685.

[3] S. Jagadeesan, V. Balasubramanian, P. Baumann, M. Neuburger, D. Haussinger, C. G. Palivan, *Inorg. Chem.*, **2013**, *52*, 12535-12544.

P30 - EXPLOITING THE SYNTHESIS AND THE ANTICHOLINESTERASE POTENTIAL OF NOVEL TYPES OF ISONUCLEOSIDES

Daniela Batista,[1] Stefan Schwarz,[2] Anne Loesche,[2] René Csuk,[2] Radek Jorda,[3] Paulo J. Costa[1] and Nuno M. Xavier[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, 5º Piso, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany;* ***(3)*** *Laboratory of Growth Regulators, Palacký University, 78371 Olomouc, Czech Republic.*

dabatista@fc.ul.pt

Modified nucleosides and nucleoside analogs have attracted considerable attention due to their propensity to display a variety of biological properties, including antiviral, antitumor, [1] antimicrobial [2] and cholinesterase inhibitory effects.[3] Regioisomers of nucleosides in which the nucleobase is linked to the carbohydrate unit at a non-anomeric position, *i.e.* isonucleosides, present better stability towards enzymatic hydrolysis that their physiological counterparts. The reported isonucleosides mostly comprise the nucleobase linked at C-2 or C-3 of furanosyl systems.[4] The access to structurally new isonucleosides and the exploitation of their biological potential remains of interest.

In this communication, the synthesis of isonucleosides embodying a nucleobase linked at C-6 of glucopyranosidyl templates is presented. A study regarding the coupling between partially protected methyl glucopyranosides and various nucleobase derivatives under Mitsunobu conditions was performed. [5,6] The outcome of the reaction was shown to be dependent on the substitution pattern of the sugar moiety when using pyrimidines, leading to uracil/thymine-linked pseudodisaccharides or to products of mono-coupling.

Biological assays revealed some compounds as good inhibitors of acetylcholinesterase (AChE) or exhibiting toxicity to tumor cells, with inhibition constants or IC_{50} values, respectively, in the micromolar concentration range. Molecular docking studies allowed inspecting the binding modes of the best AChE inhibitors to the enzyme.

The synthetic work and the findings of the biological evaluation will be revealed and discussed.

Acknowledgments: *The authors thank FCT for funding (projects IF/01488/2013/CP1159/CT0006 and UID/MULTI/00612/2013).*

References

[1] L.P. Jordheim *et al., Nat. Rev. Drug. Discov.* **2013**, *12*, 447-464.

[2] M. Winn *et al., Nat. Prod. Rep.* **2010**, *27,* 279-304.

[3] N.M. Xavier *et al., Eur. J. Org. Chem.* **2014**, *13,* 2770-2779.

[4] V. Nair. in *Recent Advances in Nucleosides: Chemistry and Chemotherapy* (Ed. C.K. Chu), Elsevier, **2002**, 149-166.

[5] N.M. Xavier *et al., Synlett*, **2015**, *26*, 2663–2672.

[6] D. Batista *et al., Pure Appl. Chem.* **2016**, *88*, 363-379.

P31 - SPIN CROSSOVER TUNING: A COMPREHENSIVE STUDY ON THE HALOGEN EFFECT

F. F. Martins,[1] A. I. Vicente,[1] L. P. Ferreira,[2,3] M. D. Carvalho,[1] V. H. N. Rodrigues,[3] M. J. Calhorda[1] and P. N. Martinho[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa,Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *BioISI, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(3)*** *Departament of Physics, University of Coimbra, 3004-516 Coimbra, Portugal*

fred.martins94@gmail.com

Technological advances have been pushing the limits of chemistry for the last few years towards creating more efficient and multifunctional molecules and materials. A phenomenon that shows great promise in molecular electronics is spin crossover (SCO).[1] This switching can be harnessed to develop materials with a wide range of possible applications such as memory or sensing nano-devices.[2] Halogen derivatized SCO molecules are of great interest as they can interact with neighboring molecules through either halogen or hydrogen bonds and additionally they can be modified through substitution or coupling reactions conferring additional properties and high versatility to the SCO molecules.[3,4]

Here we report the synthesis and characterization of halogen derivatized SCO compounds with an Fe(III) metallic center coordinated to tridentate (N2O) Schiff-base ligands. We have found that all compounds exhibit SCO with profiles ranging from gradual to abrupt with hysteresis. Detailed studies on the halogen influence on these are complemented with DFT calculations using recently developed spin state specific functionals.

Acknowledgments: *The authors thank Fundação para a Ciência e Tecnologia for financial support UID/MULTI/00612/2013, PTDC/QEQ-QIN/3414/2014 and COST Action CM1305. PNM thanks FCT for financial support (SFRH/BPD/73345/2010).*

References

[1] P. Gamez, J. S. Costa, M. Quesada, G. Aromí, Dalton Trans., **2009**, 38, 7845.

[2] J. Létard, J. Mater. Chem., **2006**, 16, 2550.

[3] E. J. Devid, P. N. Martinho, M. V. Kamalakar, I. Šalitroš, Ú. Prendergast, J. F. Dayen, ... & S. J. van der Molen, ACS nano, **2015**, 9, 4496.

[4] A. B. Gaspar, V. Ksenofontov, M. Seredyuk, P. Gütlich, Coord. Chem. Rev., **2005**, 249, 2661.

P32 - CHEMICAL STUDY OF *XIMENIA AMERICANA* L SEED OIL OBTAINED FROM FOLK AND LABORATORY PROCEDURES

Gabriel Filipe Satoto[1] and M. Eduarda M: Araújo [2]

(1) *CQB and Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Edifício C-8, 1749-016, Lisboa, Portugal*

gfsatoto@hotmail.com

Ximenia americana L. is a tropical cosmopolitan plant commonly found in Africa, India, New Zealand and Central and South America. [1] In Angola decoction of the fresh aerial part is widely used in traditional medicine for malaria control. [2] The powder obtained after drying and milling the aerial part is applied in the wounds caused by syphilis. [3] However, the oil obtained from the seeds, is the most frequent medicine. It is topically applied in treatments of the hair and to anoint the body, to moisture the skin and to protect it from solar UV radiation. [4]

This paper presents the chemical characterization of the oil obtained from the seeds of *X. americana* L., through the saponification index, the acid index, the peroxide index and the iodine index. The oil density and relative molecular mass were also determined. [5]

Two types of oil samples were studied. The first was brought from Angola, from the municipality of Bibala, located to the west of the Province of Huíla. The oil was handcrafted by woman. In the artisanal process the seeds are firstly toast, then grounded until a powder is obtained and the oil is extracted from this powder with hot water. The second sample of oil was obtained in the laboratory by Soxhlet extraction with n-pentane, from only crushed seeds.[6] Finally, the feasibility of the transformation of the artisanal *X. americana* oil to obtain a biofuel (biodiesel) was studied.

Despite the differences in color, results of the chemical characterization showed no important differences between the two samples.

Acknowledgments: *This project was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (UID/MULTI/00612/2013).*

References

[1] D. B. James, a O. Owolabi, H. Ibiyeye, J. Magaji, and Y. a Ikugiyi, "Assessment of the hepatic effects , heamatological effect and some phytochemical constituents of Ximenia americana (Leaves , stem and root) extracts," *African Joual Pharmacol.*, vol. 7, **2008** no., p. 4274-4278; [2] D. H. Feyssa, J. T. Njoka, Z. Asfaw, and M. M. Nyangito, "Uses and management of Ximenia americana, Olacaceae in semi-arid east Shewa, Ethiopia," *Pakistan J. Bot.*, vol. 44, **2012,**pp. 1177–1184; [3] U. Wölfle, "Ximenia americana L.," *Zeitschrift für Phyther.*, vol. 29, **2008**, pp. 150–153; [4] M. T. Brasileiro, "Padronização, atividade biológica e desenvolvimento de formas farmacêuticas semi-sólidas à base de Ximenia americana L.," vol. 29, 2008; [5] A. E. M. Saeed and R. S. M. Bashier, "Physico-chemical analysis of Ximenia americana . L seed oil and structure elucidation of some chemical constituents of its seed oil and fruit pulp," *J. Pharmacogn. Phyther.*, vol. 2, **2010**, pp. 49–55; [6] T. Řezanka, K. Sigler, C. O. Eromosele, and I. C. Eromosele, "Fatty acid compositions of seed oils of Haematostaphis barteri and Ximenia americana," *Phytochemistry*, vol. 68, **2002**, pp. 303–304.

P33 - HYDROMETALLURGICAL RECYCLING OF PALLADIUM: COMPUTATIONALLY SUPPORTED LIQUID-LIQUID EXTRACTION

Gonçalo Ivo Carvalho, [1] Miguel Machuqueiro[1] and Ana Paula Paiva[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa*

carvalho_@hotmail.co.uk

In this project, we plan to use computational chemistry techniques to study the preferred conformations and interactions of known thiodiglycolamides [1,2] with metallic ions. Optimization of the metal complexes structures will allow for a more effective and selective recovery of precious metal ions [3] from leaching solutions of industrial wastes. Quantum mechanics calculations will be used to determine binding affinities and interaction energies of our ligands and metal species in different media [4], including palladium, silver, titanium or aluminum. Initially, the most promising TDGA derivatives will be synthesized and tested experimentally with a focus on the loading capacity and selectivity towards Pd(II) in both model chloride solutions and in real chloride leaching aqueous phases coming from spent industrial catalysts [1-3]. The development of a method to design new organic ligands with optimized characteristics to apply in hydrometallurgical recycling processes is expected. The final goal is to recover palladium from spent industrial catalysts relying in marketable, economically profitable and environmentally friendly processes.

References

[1] A. P. Paiva, G. I. Carvalho, M. C. Costa, A. M. R. Costa, C. Nogueira, *Solvent Extraction and Ion Exchange*, **2014**, 32(1), 78-94.

[2] A. P. Paiva, O. Ortet, G. I. Carvalho, C. A. Nogueira, submitted to *Hydrometallurgy,* Jan. 2017.

[3] C. Hagelüken, *Platinum Metals Review*, **2012**, 56(1), 29-35.

[4] R. Ruhela, B. S. Tomar, A. K. Singh, R. C. Hubli and A. K. Suri, *Dalton Trans*., **2013**, 42, 7085-7091.

P34 - ANTI-INFLAMMATORY AND GASTRO PROTECTIVE ABILITY OF ALGERIAN HONEY

Hicham ZAIDI,[1] Salim Ouchemoukh[1] and Maria Eduarda Araújo[2]

(1) *Laboratory of applied biochemistry, Department of Biology Physical chemistry Faculty of Nature and Life Sciences, University of A.Mira, Bejaia 06000, Algeria;* ***(2)*** *CQB and Departamento de Quimica e Bioquimica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande Ed C8, 1749-016 Lisboa, Portugal.*

zaidi-hicham@hotmail.com

The aim of this study is to determine anti-inflammatory activity of 30 Algerian honeys. Anti-inflammatory drugs are of huge therapeutic benefit in the treatment of inflammatory diseases. The most common side effects associated with all currently available anti-inflammatory and anti-ulcerous drugs are gastrointestinal hemorrhagic, hepatotoxicity and nausea [1], which increases the interest in finding better inhibitors from natural resources. The anti-inflammatory activity of honey were examined by thermal denaturation of proteins with serum bovine albumin (BSA), according to the method described by Mizushima and Kobayashi (1964) [2]. Aqueous solution of honeys were tested at 2.5 mg/ml. The anti-inflammatory activity test shows that twenty of the honey samples, have the ability to inhibit the denaturation of the BSA, at this concentration, with values of about 45% inhibition. The different results can be explained by the diversity of the botanical origin, the nature and the contents of the active compounds such as the phenolic compounds, organic acids and enzymes [4]. Another objective was the evaluation of the gastro-protective effect. A sample of a different honey also from Algerian origin, was studied in albino mice NMRI based on the protocol reported by Hara and Okabe (1985) [3]. The mice were pre-treated with honey solution. The induction was carried out by an ethanol/HCl solution. The honey amounts used, 25, 50 and 100 mg / kg resulted in a significant effect of 57,43%, 87,90%, 83,83% inhibition. These results confirm that honey constitutes a significant source of actives substances and it has multiple therapeutic effects.

Acknowledgments: *Our warm thanks are addressed to fundação para a ciência e Tecnologia (FTC), Portugal (UID/MULTI/00612/2013). To all the members of the departments of chemistry and biochemistry for having opened their doors to us to carry out our internship within their laboratories.To all the collaborators of the laboratory of applied biochemistry and the members of the Faculty of Nature and Life Sciences, Bejaia, Algeria.*

References

[1] C. Voidarou, A. Alexopoulos, S. Plessas, A. Karapanou, I. Mantzourani, E. Stavropoulou,, K. Fotou, A. Tzora, I, Skoufos. & E. Bezirtzoglou. Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobe***, 2011,**17: 375-379.

[2] Y. Mizushima ans M. Kobayashi. *J.* Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically activeproteins. *Pharm. Pharmac.* **1964**, 20, 169-173.

[3] N. Hara and S. Okabe. Effect of gefernate on acute lesions in rats**.** *Folia Pharmacologica Japonica.* **1985,** *85,* 443-448.

[4] P.K. Bath and N. Singh**.** A comparison between *Helianthus annuus* and *Eucalyptus lanceolatus* honey. *Food Chemistry*. **1999,** 67: 389-397.

P35 - REDUCTION OF SILVER FROM DILUTE SOLUTIONS BY ELECTROLESS PRECIPITATION (RE)USING POLYANILINE FILMS

Inês J. Pereira[1] and Jorge P. Correia[1]

(1) *Centro de Química e Bioquímica, DQB, Faculdade de Ciências, Universidade de Lisboa, Portugal.*
ijordaopereira@gmail.com

It is well known that the fully oxidized state of polyaniline (pernigraniline form) reduces to produce the protonated emeraldine in acid medium at open circuit potential. It is so expected that in the presence of metallic ions that can act as oxidizing species, spontaneous deprotonation occur with simultaneous reduction of the metal ion - eventually to zero oxidation state (**Figure 1**) – continuing the process while the polymer is exposed to the solution [1]. The high electrochemical potentials of noble metals make them suitable for this electroless precipitation process in polyaniline (PAni) films [2]. This spontaneous, selective (to noble metals) and sustained reduction of metal ions is of particular importance in the field of extractive metallurgy [3].

In this work the process of electroless precipitation of silver from acidic dilute solutions of silver ions is investigated. Thin PAni films were electrochemically synthesized and exposed to 1 mM silver solutions for different periods at ambient temperature. The amount of reduced metal in each experiment was assessed by atomic absorption spectroscopy. The effect of film thickness and immersion time in the silver extraction efficiency was evaluated by optical microscopy and electrochemical characterization of the pristine films and after exposure to the silver containing solutions. The selectivity of the electroless precipitation methodology to reduce noble metal ions was also evaluated by adding significant amounts of engineering metal ions to the silver solutions. The reuse of the polymer films for multiple extraction runs was explored as well.

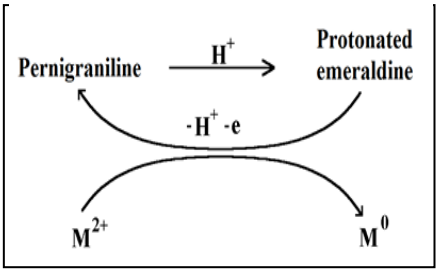


Figure 1. Electroless precipitation mechanism.

Acknowledgments: *This work is funded by FCT (Fundação para a Ciência e a Tecnologia) through the project UID/MULTI/00612/2013.*

References

[1] L. M. Abrantes, J. P. Correia, *Mat. Sci. Forum*, **1995**, *191*, 235-240.
[2] V. Lyutov, V. Tsakova, *J. Solid State Electrochem.*, **2011**, *15*, 2553-2561.
[3] Y. P. Ting, K. H. Neoh, E. T. Kang, K. L. Tan, *J. Chem. Tech. Biotechnol.*, **1994**, *59*, 31-36.

P36 - ADSORPTION OF GASES AND VAPOURS IN HYBRID SILICA XEROGELS

Joana Fernandes,[1] Jesús Echeverría[2] and João Pires[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *Departamento de Química Aplicada, Universidade Pública de Navarra.*

fc39585@alunos.fc.ul.pt

What do the plastic production from ethylene, a by-product of the oil and gas industry; the natural gas purification through the retention of carbon dioxide and the increase of the octane number by the separation of di-branched and mono-branched hexane isomers have in common? They are high commercial value processes that can use adsorption as an important unitary operation and would benefit from an innovative process involving adsorbent materials to reduce production costs and/or to improve purification of the target product.

Organic-inorganic hybrid silica xerogels are considered versatile materials and due to their composition and porosity they became promising for adsorption, separation and purification of gases and vapours. In the present work, the adsorption and separation of the systems ethane/ethylene, carbon dioxide/methane and vapours n-hexane/3-methylpentane/2,2-dimethylbutane was studied. The experiments were carried through volumetric (high pressure line) and gravimetric (microbalance) methods.

All the xerogel samples showed the capacity of separation for the pair carbon dioxide/methane, having a clear preference for carbon dioxide. For the pair ethane/ethylene only one sample showed a reasonable preference for ethylene. In order to understand said different adsorption behaviors we analyzed previous adsorption essays with N_2 and CO_2 [1] and discussed about the possible significant interactions with the gas molecules and the micropore size distribution.

Our goal is to identify the key characteristics of xerogels to maximize adsorption and selectivity. With the new acquired knowledge, new materials with enhanced properties will be easier to formulate.

Acknowledgments: *Financial help from Fundação para a Ciência e Technologia (FCT; Portugal) to CQB UID/MULTI/00612/2013 is acknowledged.*

References

[1] Moriones, P., Síntesis y caracterizacíon de xerogeles silíceos híbridos (RTEOS/TEOS; R=P,Ph). Universidade Pública de Navarra, **2015**.

P37 - NOVEL TiO_2-MoO_3 NANOCOMPOSITES FOR THE SELECTIVE OXIDATION OF BENZYL ALCOHOL TO BENZALDEHYDE

J. Diniz,[1] C.D. Nunes[1] and O.C. Monteiro[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

fc39712@alunos.fc.ul.pt

Heterogeneous photocatalysis is currently recognized as one of the most promising, advanced and environment friendly technologies due to its excellent advantages such as clean, effective, energy-saving, and low cost. Selective photocatalytic oxidation of aromatic alcohols to aldehydes is of great relevance regarding the importance of those compounds (*e.g.,* benzaldehyde) as raw materials for the synthesis of many useful chemicals, such as dyes, resins, fragrances and drugs [1].

Titanium oxide (TiO_2) has raised a great deal of interest on the scientific community due to its photocatalytic activity, chemical stability, nontoxicity and low cost. However, it presents a critical drawback: the wide band gap of TiO_2 makes only possible the use of the ultraviolet fraction of the solar light [2] (the highest and cheapest source of radiation). Therefore, great efforts have been made for improving its photocatalytic efficiency. Similarly, molybdenum trioxide (MoO_3) is also attractive due to its unique structural, electronic and optical properties. MoO_3 can present band-gap energies in the range of 2,9 to 3,1 eV and it is broadly employed in electrochromic and photochromic devices [3].

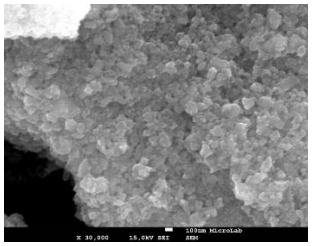


Figure 1. SEM image of TiO_2-MoO_3 nanocomposite particles.

In this study, new photocatalytic materials were prepared by combination of TiO_2 and MoO_3 particles. Nanocrystalline particles and nanocomposites were prepared using a hydrothermal approach and were subsequently characterized in terms of their structural, morphological, and electronical/optical properties.

Materials were tested in the simulated solar-light-driven selective oxidation of benzyl alcohol to benzaldehyde in acetonitrile, in which the TiO_2-MoO_3 nanocomposites exhibited higher values of selectivity and yield comparing to the isolated components.

Acknowledgments: *The authors thank Fundação para a Ciência e Tecnologia for financial support (UID/MULTI/00612/2013).*

References

[1] *C.-J. Li, G.-R. Xu, B. Zhang, J. R. Gong, High selectivity in visible-light-driven partial photocatalytic oxidation of benzyl alcohol into benzaldehyde over single-crystalline rutile TiO_2 nanorods, Appl. Catal. B-Environ.,* ***2012****, 115–116, 201–208;* [2] *M.C. Neves, J.M.F. Nogueira, T. Trindade, M.H. Mendonça, M.I. Pereira, O.C. Monteiro, Photosensitization of TiO_2 by Ag_2S and its catalytic activity on phenol photodegradation, J. Photoch. Photobio. A,* ***2009****, 204, 168–173;* [3] *Y. Zhao, J. Liu, H. Naramoto, S. Yamamoto, Preparation of MoO_3 nanostructures and their optical properties, J. Phys.-Condens. Matter,* ***2003****, 15, 547–552.*

P38 - STUDY OF ENTHALPIES OF SUBLIMATION OF ORGANOMETALLIC COMPOUNDS

Mariana T. Donato,[1] Carlos, E.S. Bernardes[1] and Manuel E. Minas da Piedade[1]

(1) *Centro de Química e Bioquímica e Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.*

marianat.donato@gmail.com

The ability to study the behaviour of a system at the molecular level to predict macroscopic properties is a long-term goal in chemistry and engineering, since it has the potential to reduce the need for expensive and time-consuming experimentation. Molecular dynamics (MD) is perhaps the most promising cost-effective computational technique to perform these studies. It is a general approach that, based on simple atom-atom pair's potential calculations, allows the investigation of many physical processes. The key aspect is the definition of an intermolecular potential function capable of accurately describing the interactions. However, although a large set of parameters exist for, e.g. hydrocarbon compounds, no reliable parametrization is available for materials containing transition metals. Thus, the work here described, is part of an ongoing project at the Molecular Energetics Group (CQB-FCUL), to produce a parametrization suitable for the study of compounds containing transition metals, by MD simulations.

One way to accurately establish interaction potentials, involves the determination of a set of parameters that reproduce the cohesive energy of materials – which can be obtained from the enthalpy of sublimation – and the spatial arrangement of the molecules (e.g. crystal structures). Although values of enthalpies of sublimation can be found in the literature, these are frequently not assigned to crystal structures, leading to large discrepancies between published data. Thus, in this work, enthalpies of sublimation of organometallic compounds were determined for well characterized materials, both in terms of chemical and phase purity. A focus was employed to compounds containing rhenium (e.g. methyltrioxorhenium and decacarbonyl-di-rhenium).

Acknowledgments: *This work was supported by Fundação para a Ciência e a Tecnologia, Portugal (Project UID/MULTI/00612/2013 and UID/QUI/00100/2013).*

P39 - ARE ABC PROTEINS A TARGET FOR RUTHENIUM-CYCLOPENTADIENYL ANTICANCER METALLODRUGS?

Patrícia Girio,[1,2] Alexis Moreno,[2] M. Helena Garcia,[1] Pierre Falson[2] and Andreia Valente[1]

(1) *Centro de Quimica Estrutural, Faculdade de Ciencias da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Drug Resistance Mechanism and Modulation Team BMSSI UMR 5086 CNRS-UCBL1, IBCP 7 Passage du Vercors 69 367 Lyon Cedex 07.*

patricia.mgirio@gmail.com

Chemotherapy is one of the basis for cancer therapy. In this frame, ruthenium compounds are nowadays seen as promising candidates due to its general low toxicity, good tolerability and stability. Our research group is engaged in the discovery of new anticancer agents with different mechanisms of action from the traditional chemotherapeutics. [1]

In this study, several complexes bearing the “Ru-cyclopentadienyl bipyridine” core have been carefully selected among a large family of compounds. All the compounds display a cytotoxic effect against human cancer cells, with different modes of action. Thus our goal was to evaluate the multidrug resistance (MDR) response that they may trigger upon addition. Specifically, we explored the possibility for them to be exported by the main MDR ABC exporters. These proteins are key players in this phenotype (**Figure 1**). They are overexpressed in cancer cells when stressed by cytotoxic compounds. These proteins are addressed to the plasma membrane and actively pumping drugs out of the cells. Their action decreases the intracellular drug concentration below its toxicity threshold. They also confer the same resistance to other drugs as these pumps are multispecific. [2]

We will present the results obtained for our ruthenium complexes in different human cells transfected with each MDR pump, ABCB1, ABCC1, ABCC2 and ABCG2, using flow cytometry alone or coupled to mass spectrometry (CyTOF technology). To determine the affinity of those compounds with the MDR pumps and to predict the preferred structure, we also carried out molecular docking on x-ray structures and 3D models.

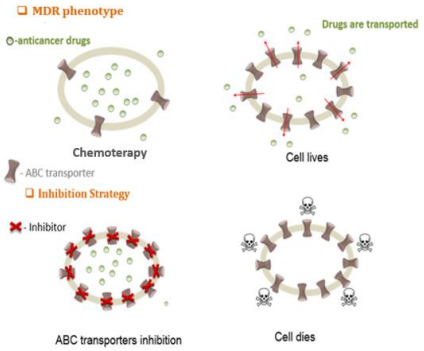


Figure 1. Drug resistance phenotype.

Acknowledgments: *This work was financed by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia, FCT) within the scope of project UID/QUI/00100/2013. Andreia Valente acknowledges the COST action CM1302 (SIPs), the Investigator FCT2013 Initiative for the project IF/01302/2013 (acknowledging FCT, as well as POPH and FSE - European Social Fund). For all the help and assistance Patrícia Gírio wants to thank Rachad Nasr, Josiane Kassis, Nathalie Guragussian and Clarisse Faugier.*

References:

[1] Asa S. L., Ezzat S., The Annual Review of Pathology, 2009, 4, 97-126; [2] E. Baiceanu, KA. Nguyen, L. Gonzalez-Lobato, R. Nasr, H. Baubichon-Cortay, F. Loghin, et al. 2-Indolylmethylenebenzofuranones as first effective inhibitors of ABCC2. Eur J Med Chem. 2016;122:408-18.

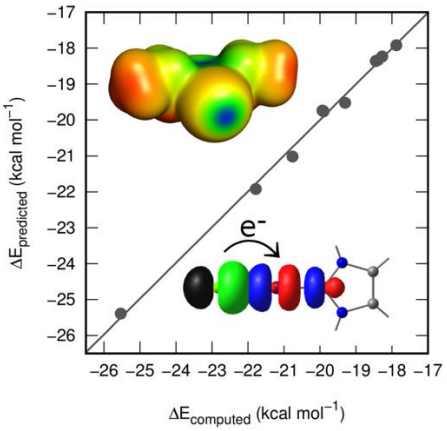
P40 - TUNING ION-PAIR HALOGEN BONDS TOWARDS EFFICIENT ANION RECEPTORS IN SOLUTION

Rafael Nunes[1] and Paulo J. Costa[1]

(1) *Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

rsnunes@fc.ul.pt

Halogen bonds (XB) are highly directional, attractive interactions involving a halogen atom (X) and a Lewis base (B), in a complex type R–X···B (X = Cl, Br or I). The nature of this specific type of non-covalent interaction has been predominantly explained by the existence of a localized electrophilic region at X, named σ-hole, while evidence for significant contributions from charge-transfer have been the subject of intense discussion recently. XBs have found widespread application, amongst other fields, in anion recognition in solution.[1] In particular, the charged haloimidazolium or halotriazolium motifs are shown to establish very strong XBs with anions in competitive aqueous media. In this communication, we investigate this class of ion-pair systems by quantum mechanical methods discussing the key roles of solvent and substituents on the XB nature and strength, and their implications for the design of efficient anion receptors working in solution. [2]



Acknowledgments: *The authors thank Fundação para a Ciência e a Tecnologia (FCT), Fundo Social Europeu, and Programa Operacional Potencial Humano for the Investigador FCT contract and project IF/00069/2014. FCT is also acknowledged for financial support through strategic project UID/MULTI/00612/2013.*

References

[1] a) T. M. Beale, M. G. Chudzinski, M. G. Sarwar, M. S. Taylor, *Chem. Soc. Rev.* **2013**, *42*, 1667–1680; b) A. Brown, P. D. Beer, *Chem. Comm.* **2016**, *52*, 8645–8658.

[2] R. Nunes, P. J. Costa, *Chem. Asian J.* **2017**, *accepted article*, 10.1002/asia.201601690.

NEW RUTHENIUM-METHYLCYCLOPENTADIENYL FAMILY OF ANTICANCER AGENTS: SYNTHESIS, CHARACTERIZATION AND *IN VITRO* STUDIES

Ricardo Teixeira,[1] Leonor Côrte-Real,[1] Fernando Avecilla,[2] M. Helena Garcia,[1] Fernanda Marques[3] and Andreia Valente[1]

(1) *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Departamento de Química Fundamental, Universidade da Coruña, Campus de A, Zapateria 15071, A Coruña, Spain;* ***(3)*** *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, E.N.10, 2695-066 Bobadela LRS, Portugal.*

ricardojt2009@gmail.com

Although the death rates from cancer, the second leading cause of death worldwide, have decreased since the early 90's [1] there is still an outrageous number of lives that are lost due to this condition. Ruthenium-based complexes have emerged a few years ago as a promising alternative to the traditional therapy based on platinum chemotherapeutics.

In the last few years, our research group focused special attention on the piano-stool family of compounds that were screened for their chemotherapeutical potential. Most of the compounds showed significant cytotoxicity against several cancer cell lines, surpassing many times cisplatin. [2,3]

In this context, a novel family of Ru^{II}-methylcyclopentadienyl compounds of general formula $[(\eta^5\text{-MeCp})Ru^{II}(NN)PPh_3)]^+$ (MeCp = methylcyclopentadienyl; NN = bipyridine, bpy, and its derivatives), isolated as $CF_3SO_3^-$ salts, was prepared and characterized by spectroscopic and analytical techniques. A bipyridine-based macromolecular ligand, obtained by ring-opening polymerization of D,L-lactide, was also coordinated to the ruthenium center, originating a polymer-metal conjugate. In this case a better cellular internalization and improvement on the selectivity to cancer cells relatively to low molecular weight drugs is envisaged. [3] The anticancer activity of all complexes was screened against cancer cells.

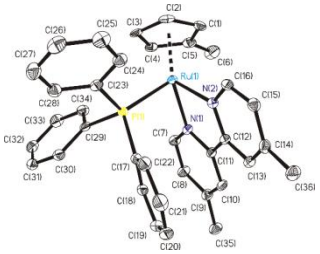


Figure 1 – Molecular structure of complex $[Ru(MeCp)(Me_2bpy)(PPh_3)]^+$, showing the crystallographic labeling scheme. Hydrogen atoms are omitted for clarity.

Acknowledgments: *This work was financed by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia, FCT) within the scope of project UID/QUI/00100/2013. Andreia Valente acknowledges the COST action CM1302 (SIPs), the Investigator FCT2013 Initiative for the project IF/01302/2013 (acknowledging FCT, as well as POPH and FSE - European Social Fund).*

References

[1] R. L. Siegel, K. D. Miller and A. Jemal, Cancer Statistics, 2017, CA: A Cancer J Clin 2017, Jan 5, doi:10.3322/caac.21387.

[2] T. S. Morais, A. Valente, A. I. Tomaz, F. Marques, M. Helena Garcia, Future Med.Chem.2016, 8(5), 527-544.

[3] A. Valente, M.H. Garcia, F. Marques, Y. Miao, C. Rousseau, P. Zinck, J. Inorg. Biochem. 2013, 127, 79-81.

P42 - NEW RUTHENIUM(II) COMPLEXES COMPRISING CARBOHYDRATES FOR CANCER THERAPY: SYNTHESIS AND CHARACTERIZATION

Miguel de Freitas,[1] Tânia Morais,[1,2] Nuno Xavier[3] and M. Helena Garcia[1]

(1) *Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Avenida Professor Egas Moniz, 1649-028 Lisboa, Portugal.;* ***(3)*** *Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.*

mike.sbm.freitas@gmail.com

As one of the leading causes of death in the world, it is necessary to find more efficient and selective drugs for cancer therapy. Since it was proved that half "sandwich" Ru(η^5-C_5H_5) derived compounds have, in the most cases, a better cytotoxicity, against several cancer cell lines, than that of cisplatin (*cis*-[$Pt(Cl)_2(NH_3)_2$]), this topic has been hugely developed during the recent years. [1]

Carbohydrates are an excellent class of tunable ligands for use in medicinal inorganic chemistry, because of their role in glycobiology, which leads to reduced toxicity and an improved solubility and molecular targeting, when attaching them to a metal leads. Its proven that modified carbohydrates can interfere with carbohydrate–protein interactions and can inhibit cell–cell recognition and adhesion phenomena, essential processes in cancer growth and progression. [2]

Due to the high energy demand of developing tumors, which can only be satisfied by glycolysis, coordinating derivatives of carbohydrates to metal complexes may improve the cytotoxic potential of the new compounds by increasing the selectivity to cancer cells. [2]

In this context, the work presented here was intended to reach a new family of ruthenium complexes through the linking of sugar derivatives with ruthenium center combining the good results of Ru(η^5-C_5H_5)-based complexes with the promising features that carbohydrates can play in bioorganometallic chemistry. The synthesis and characterization of new piano stool "RuCp" compounds with carbohydrate and N-heteroaromatic ligands will be disclosed herein (**Figure 1**).

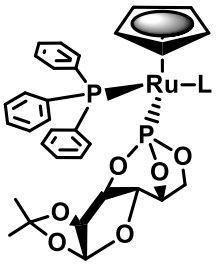


Figure 1. General structure of the coordination compound with L= chlorine or N-heteroaromatic ligands.

Acknowledgments: *Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia, FCT) for funding through the FCT Investigador program (N.M. Xavier), SFRH/BPD/93513/2013 post-doctoral Grant (T.S. Morais) and financial support (UID/QUI/00100/2013 and UID/MULTI/00612/2013).*

References

[1] T. S. Morais; A. Valente; A. I. Tomaz, M. H. Garcia, Future Med. Chem., **2016**, 8(5), 527–544.

[2] M. Hanif, A. A. Nazarov, A. Legin et al. Chem. Commun., 2012, 48, 1475–1477.

P43 - BAR ADSORPTIVE MICROEXTRACTION – SCREENING OF SYNTHETIC CANNABINOIDS IN ORAL FLUIDS

S.M. Ahmad[1], N.R. Neng[1], H.M. Gaspar[1] and J.M.F. Nogueira[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal*

smahmad@fc.ul.pt

The last decade has seen an increase of new psychoactive substances (NPS) that were been widely spread through "smart shops" and over the Internet. NPS are psychoactive substances not internationally controlled but may pose a similar risk to traditional drugs. Synthetic cannabinoids are one of most diffused NPS, since they are legal alternatives of *cannabis*. Since 2008, 160 new synthetic cannabinoids were detected in a wide range of different products, of which 24 were reported in the last year. Many toxicity symptoms were associated with the consumption of these drugs: anxiety, paranoia, tachycardia, irritability, hallucination, numbness, seizures, high blood pressure, drowsiness, slurred speech and in some cases even death [1].

For this reason, there is the need for innovative and alternative analytical approaches that allow an effective monitoring of these compounds in biological matrices, in particular using non-invasive sampling, *e.g.* oral fluids. In this contribution we present the development, optimization, validation and application of a novel methodology, based on bar adsorptive microextraction (BAμE) [2], followed by microliquid desorption, in combination with high performance liquid chromatography with diode array detection for monitoring eight synthetic cannabinoids (AM-694, SGT-25, MAM-2201, 5F-UR-144, JWH-018, JWH-122, UR-144 and AKB-48) in oral fluids. This new approach presents excellent extraction yields (50 - 80 %) with limits of detection in between 2.0 and 6.0 μg L^{-1}, that proved to be an effective methodology for screening these compounds in oral fluids.

Acknowledgments: *The authors thank Fundação para a Ciência e a Tecnologia (Portugal) for financial support through project UID/MULTI/00612/2013, as well as the Post-Doc (SFRH/BPD/86071/2012) and PhD grants (SFRH/BD/107892/2015). The authors thank the toxicology sector of the "Laboratório de Polícia Científica da Polícia Judiciária" for providing the synthetic cannabinoids products, within the protocol established with the "Faculdade de Ciências da Universidade de Lisboa".*

References

[1] European Monitoring Centre for Drugs and Drug Addiction, *European Drug Report 2016: Trends and Developments*, Publications Office of the European Union, Luxembourg, **2016**.

[2] N.R. Neng, A.R. Silva, J.M.F. Nogueira*, J. Chromatogr. A* **2010**, *1217*, 7303-7310.

P44 - PAIR QUALITY ASSESSMENT IN SOME AREAS OF C8 BUILDING

Sara Santos[1] and Cristina Oliveira[1]

(1) *Centro de Química Estrutural (CQE), Departamento de Química e Bioquímica da Faculdade de Ciências da Universidade de Lisboa.*

isasantos_92@hotmail.com

The environment inside a building is a complex system which involves several parameters that have impact on the health and comfort of its users. Several areas can differ on environmental conditions. Air transferred between neighbour divisions or outdoor are limited by the building structure and operational and use strategies (mechanical ventilation, natural ventilation, etc.)

The growing concerns about indoor air quality (IAQ), the maintenance-free air conditioning systems and their relationship with health problems led to the establishment of legal requirements related to building IAQ, which were published in DL. n. º 79/2006. This law demands the hygienic inspection of HVAC systems, the verification of the maintenance plan and the monitoring of the following pollutants: particulate matter (PM_{10} and $PM_{2.5}$), CO_2, CO, O_3, formaldehyde, volatile organic compounds (VOCs), bacteria, fungi and radon.

There is good evidence of the effects of short-term exposure to PM_{10} on respiratory health, but for mortality, $PM_{2.5}$ is a stronger risk factor than PM_{10}. PM analysis is very important since it is estimated that approximately 3% of cardiopulmonary deaths and 5% of lung cancer deaths are globally due to them. The exposure by inhalation reduces life expectancy of the populations which can rise about 8.6 months in the regions with high levels of PM. [1]

In this work PM_{10} and $PM_{2.5}$ are collected in the 5th floor of FCUL C8 building, in two chemistry laboratories, and in one of the corridors, which is a common area to all those who use that floor. Samples will be analysed for inorganic and organic composition.

Acknowledgments: *The authors acknowledge FCT, for financial support through UID/QUI/00100/2013 project.*

References

[1] WHO Regional Office for Europe, “Health effects of particulate matter”, Ed. **2013**, 2-7.

P45 - SYNTHESIS AND BIOLOGIC EVALUATION OF PSYCHOACTIVE CATHINONES

Soraia Bronze,[1] Susana Santos[1], Rita Pacheco[1,2] and Helena Gaspar[1,3]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa;* ***(2)*** *Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Av. Conselheiro Emídio Navarro, 1959-007 Lisboa;* ***(3)*** *MARE-Centro de Ciências do Mar e do Ambiente, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa.*

soraiarsb@live.com.pt

New psychoactive substances (NSP) are narcotic or psychotropic compounds not internationally controlled, but are consider potentially dangerous like the drugs listed in the 1961 and 1971conventions. More than 560 novel NPS were reported since 2005, 103 of which are synthetic cathinones [1]. These compounds are β-keto phenethylamines derivatives, structural analogues of the natural-occurring *S*-cathinone from the khat plant. Its neuropharmacological effect is similar to some illegal drugs such as MDMA and amphetamine, which includes the inhibition of the monoamine transporters, like SERT, NET and DAT, although the interaction with each transporter depends on the structure [2,3].

Several reported studies associate the consumption of cathinones with the growth of severe hepatic injury cases, speciality in countries were khat consumption is legal [2]. Additionally there has been an increase in incidents, or even deaths, associated with cathinone consumption. Since there are only a few reported studies on hepatotoxicity of cathinones [4,5], the synthesis of 8 cathinones (methcathinone, buphedrone, pentedrone, mephedrone, 4-MEC, α-PPP, α-PBP and α-PVP) was performed in order to evaluate its cytotoxicity against human hepatoma cell line Hep G2.

All the tested cathinones showed to be hepatotoxic with EC50 ranging from 1.28 to 6.24 mM, being mephedrone the most toxic and α-PPP the less. The results suggest that cathinones toxicity increase with the aliphatic chain length and with the presence of a methyl group in the aromatic ring. These studies and further on going results will clarify the relation between structure-activity of cathinones.

Acknowledgments: *The authors acknowledge FCT for financial support through the project UID/MULTI/00612/2013. This work was done within the protocol between Faculdade de Ciências da Universidade de Lisboa and the Laboratório de Policia Cientifica da Policia Judiciária.*

References

[1] EMCDDA-Europol, EU Drug Markets Report: In-Depth Analysis, EMCDDA–Europol Joint publications, Publications Office of the European Union, Luxembourg, **2016**.

[2] F. Pantano et al, *Int. J. Mol. Sci*. **2016,** 17, 580.

[3] J. DeRuiter et al, *J. Chrom. Science*, **1994**, 552-564.

[4] H. Gaspar et al, *Forensic Sci Int*, **2015**, 252, 168-176.

[5] M. J. Valente et al, *Toxicol Sci*, **2016**, 1-14.

P46 - ZNO-BASED NANOSTRUCTURES FOR PEROVSKITE SOLAR CELL APPLICATIONS: PREPARATION, CHARACTERIZATION AND APPLICATION

T. Frade,[1,2] K. Lobato,[2] R. Tena-Zaera[3] and A. Gomes[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(2)*** *Instituto Dom Luiz, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;* ***(3)*** *IK4-CIDETEC, Parque Tecnológico de San Sebastián, Pº Miramón, 196, 20009 Donostia-San Sebastián (Gipuzkoa), Spain.*

tcfrade@fc.ul.pt

In the field of halide perovskite solar cells, such as $CH_3NH_3PbI_3$ one of the most commonly used, developing low temperature solution processable electron selective contacts (ESCs) is of high interest in order to capitalize the ability of the halide perovskites to obtain high quality films at soft processing temperatures making possible to fabricate plastic-based flexible photovoltaic devices. Among the different ESC options, perovskite solar cells based on ZnO have shown competitive power conversion efficiencies (i.e. > 15 % [1,2]).

Several publications describe 1D ZnO nanostructures such as nanorods, as a material that provide direct and continuous pathways for charge transport. ZnO nanorods can be easily grown by different physical and chemical methods, being electrochemical deposition probably the most promising one for such purposes because it is easy to scale-up, low-cost and ecofriendly [3].

Coupling the ZnO nanostructures with another material to form a composite, like core-shell materials, a variety of properties different from the original ZnO emerge. One such proposed structure is the coverage of ZnO nanorods with a thin TiO2 layer that has similar properties. The objective in this case is to benefit from the conjugation of the efficient electron transport in ZnO with the chemical stability and lower density of recombination defect states of TiO_2 [4].

In this work we present ZnO nanorods cores prepared by pulsed electrochemical deposition, coated with TiO_2 shell obtained by solvothermal method, where several conditions were tested and their effects on the resultant films were characterized by morphological, structural, optical, and photoelectrochemical response.

Acknowledgments: *This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (Projects UID/MULTI/00612/2013 and PhD grant SFRH/BD/84669/2012). The authors are grateful to Dr. T Monteiro group (Departamento de Física e I3N, Universidade de Aveiro) for the assistance with Raman measurements, to Msc. Daniel Siopa (CQB-FCUL, Universidade de Lisboa) for the EIS measurements, and Dr. Ivet Kosta (IK4-CIDETEC) for the assistance with solar cell measurements.*

References

[1] D. Y. Liu, T. L. Kelly, *Nature Photon* **2014**, *8,* 133-138.

[2] J. You, L. Meng, T-B. Song, T-F. Guo, Y. Yang, W. H. Chang, Z. Hong, H. Chen, H. Zhou, Q. Chen, Y. Liu, N. de Marco, Y. Yang, *Nature Nanotechn.* **2016**, *11*, 75-81.

[3] C. Z. Yao, B. H. Wei, P. Zhang, X. H. Lu, P. Liu, Y. X. Yong, *J. Rare Earths* **2011**, *29*, 133-137.

[4] M. Law, L. E. Greene, A. Radenovic, T. Kuykendall, J. Liphardt, P. Yang, *J. Phys. Chem. B* **2006**, *110*, 22652-22663.

P47 - NANOSTRUCTURED PLATFORMS FOR SENSITIVE IMMUNOSENSORS

Telmo O. Paiva,[1] Inês Almeida,[1] Joaquim T. Marquês,[1] Maria L. Oliveira,[1] Maria D. Carvalho,[1] Wei Liu,[2] Yu Niu,[2] Gang Jin[2] and Ana S. Viana[1]

(1) *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;* ***(2)*** *NML, Institute of Mechanics, Chinese Academy of Sciences, Beijing, 100190, China.*

tmpaiva@fc.ul.pt

The quantification of target analytes, usually present in trace amounts in clinical samples requires methods with high detection limits [1]. By using the natural antibody-antigen affinity, immunosensor platforms are a useful approach that combines stability, biocompatibility, versatility with high sensitivity [2]. One of the most popular approaches to improve the detection limit of optical immunosensors, such as those using the surface plasmon resonance (SPR), is the incorporation of nanoparticles (*e.g*. Au, Fe_3O_4) [3]. Considering the sandwich-type immunosensors, where small target molecules can be detected using a couple of antibodies, the nanoparticles can be used for instance to i) to increase the amount of the immobilized primary antibody, and ii) to label the secondary antibody employed on the detection layer (*e.g*. change of the optical properties). In this work, we are developing innovative strategies to tackle both challenges in the biorecognition and the detection layers, by combining adequate nanoparticle biofunctionalization methodologies with *in situ* dithiocarbamate chemistry [4]. Apart from the optical detection techniques (SPR, conventional and imaging ellipsometry) to follow the antibody-antigen reaction, we are also using electroactive molecules (*e.g.* porphyrin, ferrocene) to label gold and Fe_3O_4 nanoparticles in order to amplify the detection limits by using electrochemical techniques.
Overall, we aim to develop a useful approach that can be tailored for the sensitive detection of cancer biomarkers in clinical trials or small pollutants (*e.g.* toxins) in environmental samples.

Acknowledgments: *Financial support by FCT: PhD scholarship (SFRH/BD/70673/2010) and projects: PEst 2015-2020-UID/Multi/00612/2013, IF/00808/2013 (POPH, UE-FSE), 7th Sino-Portugal Scientific and Technological Cooperation 2013-2015 and PTDC/CTM-NAN/0994/2014.*

References

[1] X. Liu, Q. Dai, L. Austin, J. Coutts, G. Knowles, J. Zou, H. Chen, Q, Huo, *J. Am. Chem. Soc.* ***2008***, *130*, 2780-2782.
[2] I. Almeida, J. T. Marquês, W. Liu, Y. Niu, R. F. M. de Almeida, G. Jin, A. S. Viana, *Colloids Surf., B* ***2015****, 136,* 997-1003.
[3] Y. Uludag, I.E. Tothill, *Anal. Chem.* ***2012*****,** *84*, 5898-5904.
[4] I. Almeida, F. Henriques, M. D. Carvalho, A. S. Viana, *J. Colloid Interface Sci.* ***2017****, 485*, 242-250.
[5] Y. Niu, A. I. Matos, L. M. Abrantes, A. S. Viana, G. Jin, *Langmuir* ***2012****, 28*, 17718-17725.

P48 - STUDIES ON WITTIG OLEFINATION TOWARDS BRANCHED CHAIN SUGARS

Vasco Cachatra,[1] Rafael Nunes,[1] Diogo Vila-Viçosa,[1] Maria José Calhorda[1] and Amélia P. Rauter[1]

(1) Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, Piso 5, Campo Grande, 1749-016 Lisboa, Portugal.

vmcachatra@fc.ul.pt

Branched chain sugars are versatile scaffolds that are often used as precursors of atypical structures, for instance those sugars embodying a tetrahydrofuran ring fused to a pyranose. These compounds are key intermediates in the synthesis of miharamycins and related nucleosides, that exhibit diverse bioactivities, namely antifungal activity against the rice blast disease fungus Pyricularia oryzae, [1] or selective butyrylcholinesterase inhibition. [2] This enzyme plays an important role in later Alzheimer's disease (AD) stages and is also present in AD senile plaques.

Several methodologies have been reported for the synthesis of branched chain sugars using the Wittig reaction as a key step, but the main disadvantage relies with the stereocontrol of the resulting alkene. [3] In this work we present an easy to run synthetic strategy based on sugar protection and solvent choice to provide a stereo- and regiocontrol of the Wittig reaction. DFT studies were carried out to understand the stereochemistry of the alkene formed. The Wittig product with the appropriate stereochemistry is used as the precursor for the new synthesis of the bicyclic miharamycins sugar moiety. [4]

Acknowledgments: *Fundação para a Ciência e a Tecnologia is gratefully acknowledged for financial support of the project UID/ MULTI/00612/2013 and for Ph.D. grant for Vasco Cachatra. (SFRH/ BD/90359/2012).*

References

[1] F. Marcelo, J. Jiménez-Barbero, J. Marrot, A. P. Rauter, P. Sinaÿ, Y. Blériot, *Chem. Eur. J.* **2008**, *14*, 10066-10073.

[2] F. Marcelo, F. M. S. Silva, M. Goulart, J. Justino, P. Sinaÿ, Y. Blériot, A. P. Rauter, *Bioorg. Med. Chem.* **2009**, *17*, 5106-5116.

[3] V. Cachatra, A. P. Rauter, *Curr. Org. Chem.* **2014**, *18*, 1731-1748.

[4] V.Cachatra, A. Almeida, J. Sardinha, S. D. Lucas, A. Gomes, P. D. Vaz, M. H. Florêncio, R. Nunes, D. Vila-Viçosa, M. J. Calhorda, A. P. Rauter, *Org. Lett.* **2015**, *17(22)*, 5622-5625.

Credits

Logo design: Ana Marta de Matos

Website design and management: Leonor Côrte-Real

Book of Abstracts design: Samir Marcos Ahmad

Sponsors

Ciências ULisboa
Faculdade de Ciências da Universidade de Lisboa

U LISBOA | UNIVERSIDADE DE LISBOA

Hovione

sumol+compal

SAPEC AGRO BUSINESS

Associação de Trabalhadores
1986 - 2016
30 anos a criar laços

SRQ SOQUÍMICA

solítica
SOLUÇÕES ANALÍTICAS ADEQUADAS ÀS NECESSIDADES DOS NOSSOS PARCEIROS.

Pastéis de Belém
≈ desde 1837 ≈

Caixa Geral de Depositos

paralab

LABOR SPIRIT, Lda.

SOCIEDADE PORTUGUESA DE QUÍMICA

http://chembiochem.campus.ciencias.ulisboa.pt