# Book of Abstracts

## 2023

**3rd Chem & Biochem Students Meeting**

**JULY 13th**

**3rd ChemBiochem Students Meeting**

# 3rd Chem & Biochem Students Meeting

# Book of Abstracts

**July 13th, 2023**

**Faculdade de Ciências da Universidade de Lisboa**

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# Welcome Message

The Organizing Committee is pleased to welcome you to the 3rd edition of the Chemistry and Biochemistry (Chem & Biochem) Students Meeting of the Departamento de Química e Bioquímica (DQB) from Faculdade de Ciências da Universidade de Lisboa.

The main goal of this event is to ignite scientific creativity and foster the exchange of ideas among students from DQB and other institutions. We also aim to promote camaraderie and multidisciplinary collaboration by establishing new research partnerships.

The program features engaging oral, flash, and poster presentations by talented PhD, MSc, and BSc students in the fields of Chemistry and Biochemistry, who will have the opportunity to share and discuss their groundbreaking research with their peers and experienced researchers/professors. To recognize excellence, prizes will be awarded to the best presentations, determined by a scientific jury consisting of 11 esteemed professors from DQB.

But that's not all! Alongside the regular presentations, we have arranged two captivating plenary lectures by renowned researchers who are pushing the boundaries of chemistry and biochemistry. Additionally, there will be a thought-provoking Round Table discussion on the Science beyond academia: Entrepreneurship – an essential and trending topic in our society nowadays.

Get ready for an eventful and inspiring experience!


The Organizing Committee wishes all participants a very fruitful meeting!


Lisbon, July 13th, 2023
The Organizing Committee
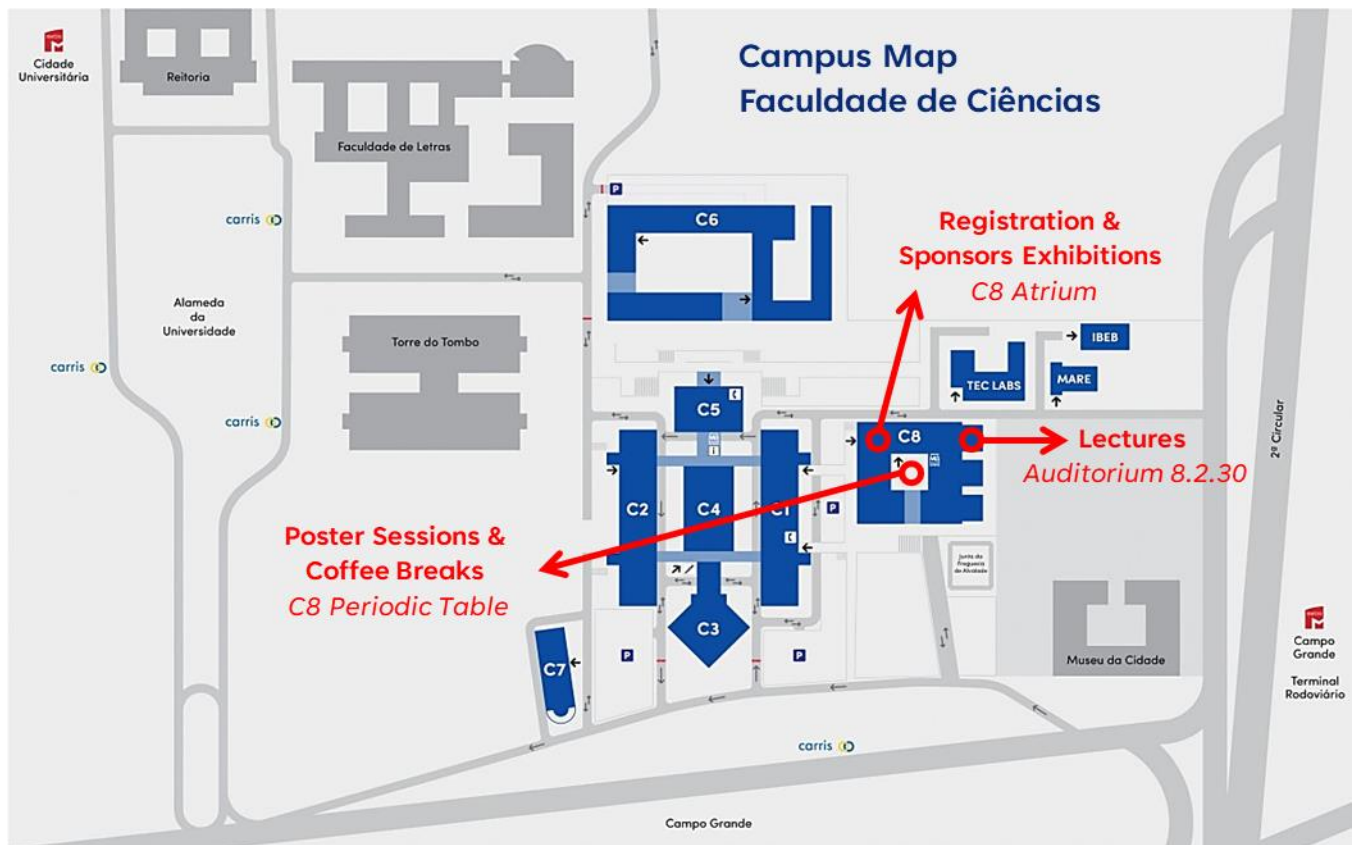
# Venue

**Registration:** C8 Atrium

**Sponsors Exhibitions:** C8 Atrium

**Opening, Closing and Awards Sessions:** C8 Auditorium 8.2.30

**Plenary Lectures, Oral and Flash Communications:** C8 Auditorium 8.2.30

**Poster Sessions:** C8 Periodic Table

**Coffee Breaks:** C8 Periodic Table



**How to arrive by:**

Bus: Campo Grande (206, 207, 701, 717, 736, 750, 767) or Cidade Universitária (731, 735, 738, 754)

Subway: Campo Grande (yellow/green lines) or Cidade Universitária (yellow line)

Car: 2ª circular road, Campo Grande/Cidade Universitária exit.

## 3rd ChemBiochem Students Meeting

13th July 2023 | Faculty of Science – University of Lisbon (C8)

# Program

### Morning Session

**8:00-9:00** Event Registration

**9:00-9:15** Formal Opening
Prof. Manuel Minas da Piedade – President of Chemistry and Biochemistry Department
Prof. Pedro Almeida – Subdirector of Faculty of Science of University of Lisbon
Prof. Vasco Bonifácio – Portuguese Society of Chemistry (SPQ)

**9:15-10:15** Plenary Lecture I – Dr. Pedro Mateus (ITQB NOVA) | Chair: Nuno Galamba
*Foldamers: privileged structures for molecular recognition and biological applications*

**10:15-11:00** Coffee break + Poster Session I

**11:00-12:00** Oral Session I | Chairs: Carlos Bernardes & Marta Silva
O1. Ana Furtado *Development of Metal-based Molecularly Imprinted Polymers in Supercritical $CO_2$ for Biorecognition Applications*
O2. António Figueira *Inhibition of Aβ42 oligomers relevant in Alzheimer's disease by a chaperone multimer*
O3. Andreia Fortuna *Describing halogen anisotropy in solvation, membrane permeability, and protein-ligand systems: towards better drugs*
O4. Fernanda Murtinheira *Proteomic profiling of astroglial and microglial cell models of the Autosomal Recessive Ataxia of Charlevoix-Saguenay (ARSACS)*

**12:00-13:00** Flash Pitches | Chairs : Sara Ferreira & António Figueira
F1. Beatriz Machado *Synthesis and Characterization of Copper (I) complexes and study of their catalytic activity in benzyl alcohol oxidation*
F2. Beatriz Farinha *Unveiling the Functional Role of TGR5 in Fat-to-Liver Communication: Discovery of Novel Agonists*
F3. Duarte Borralho *Electrocatalytic Ammonia Conversion Using Metal-Organic Frameworks Films*
F4. João Miranda *Understanding Metastasis Organotropism Patterns Through Within-cell and Between-cells Molecular Interaction Networks*
F5. Ali Hassan *Photocatalytic nanocomposite for emerging pollutants remediation*

F6. João Saavedra *Scalable production of bacteriophages preparations*
F7. Henrique Costa *Biomass-derived activated carbons for point-of-use water treatment*
F8. Mariana Alves *The role of RIPK3 in the crosstalk between hepatocytes and macrophages in experimental models of NAFLD*
F9. Jéssica Cerqueira *Analysis of biogenic volatile organic compounds in Mediterranean shrubs using headspace-bar adsorptive microextraction (HS-BAμE)*
F10. Raquel Torres *Exploring Epithelial Mesenchymal-Transition (EMT)-associated factors in Cystic Fibrosis*

**13:00-14:15** Lunch break

### Afternoon Session

**14:15-15:15** Plenary Lecture II – Dr. Liana Silva (iMed.ULisboa) | Chair: Ana Coutinho
*Fluorescence-based strategies to study the biophysical properties of biological membranes and organelles*

**15:15-16:15** Oral Session II | Chairs: Bárbara Henriques & Jaime Coelho
O5. João Franco Machado *Innovative smart metallodrug delivery systems for targeted therapy of metastatic breast cancer*
O6. Francisco Traquete *Generative Adversarial Networks for untargeted metabolomics data augmentation, friend or foe?*
O7. Rita Lopes *Synthesis of psychoactive cathinones and its metabolites: the role of metabolism*
O8. João Vitorino *Into the Early Stages of Protein Aggregation: How Monomers generate Polymers*

**16:15-17:00** Coffee break + Poster Session II

**17:00-18:15** Round Table

#### Science beyond academia: Entrepreneurship
Moderator: Rita Tomé Rocha (Tec Labs)
Ricardo Perdigão Henriques (Bionova Capital)
Miguel Prudêncio (iMM)
Maria Helena Garcia (R-nuucell)

**18:15-18:25** Hovione

**18:25-18:40** Closing Session & Awards

# Plenary

# Lectures

# PL1. Foldamers: privileged structures for molecular recognition and biological applications

Mateus, Pedro (1)

(1) Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

E-mail: pmateus@itqb.unl.pt

Folding of molecular strands is the method selected by nature to accurately organize chemical groups in space and endow biopolymers with fascinating functions such as molecular recognition, catalysis, and information storage and transfer.

In the past 20 years, chemists have developed molecules with a propensity to fold into well-defined structures, termed foldamers,[1] which allowed the creation of either internal or external molecular surfaces predisposed to intermolecular recognition.

In this talk, I will give an overview of my experience with this class of molecules and of on-going projects in my lab that seek to use foldamers in biological contexts.
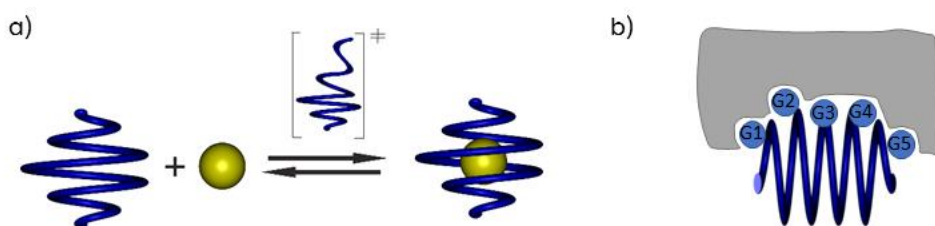


**Fig. 1.** Molecular recognition using foldamers: endomolecular (a) and exomolecular (b) recognition.

**References:** [1] a) Gellman, S H (1998) Foldamers: A manifesto. Acc. Chem. Res., 31, 173–180; b) Hill, D J et al. (2001) A Field Guide to Foldamers. Chem. Rev., 101, 3893-4012; b) Huc, I. (2004) Aromatic oligoamide foldamers. Eur. J. Org. Chem., 17–29.

# PL2. Fluorescence-based strategies to study the biophysical properties of biological membranes and organelles

Ventura, AE (1,2,3); Pokorna, S (4); Carreira, AC (1,5); Futerman, AH (3); de Almeida RFM, Prieto M (3,6); Silva, Liana C (1)

(1) iMed.ULisboa - Research Institute for Medicines, Faculdade de Farmácia, UL, Lisboa, Portugal
(2) iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, UL, Lisboa, Portugal
(3) Weizmann Institute of Science, Department of Biomolecular Sciences, Rehovot, Israel
(4) J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences Czech Republic
(5) CQB - Centro de Química e Bioquímica, Faculdade de Ciências, UL, Lisboa, Portugal
(6) Associate Laboratory i4HB - Institute for Health and Bioeconomy at Instituto Superior Técnico, UL, Lisboa, Portugal
E-mail: lianacsilva@ff.ulisboa.pt

Biological membranes exhibit an intricate organization into structurally and functionally distinct regions. This complexity is maintained by specific lipid and protein interactions and governs cell response to stimuli. Beyond their structural role, membrane lipids play crucial functions in cell processes. It is hypothesized that the biological functions of bioactive lipids are mechanistically linked to their ability to modulate biophysical properties like membrane fluidity, lateral organization, and permeability, as well as their ability to undergo morphological alterations. Impaired lipid metabolism, pathological conditions, stress stimuli, and membrane-drug interactions all contribute to alterations in membrane lipid composition and properties, strongly affecting membrane function. Despite their importance, investigating lipid interactions in biological membranes poses challenges due to their inherent complexity. To overcome this challenge and to specifically address lipid-lipid interactions in complex systems, we established a bottom-up experimental approach that starts with the development of artificial systems mimetic of biological membranes and organelles. We took advantage of these controlled and reproducible systems to obtain the photophysical fingerprints of multiple fluorescent probes, creating a unique toolbox able to identify specific alterations in membrane properties. Our approach, integrated with multiple fluorescence-based methodologies, such as spectroscopy, microscopy, and fluorescence lifetime imaging microscopy (FLIM), enables the evaluation of the impact of lipids on living cell membrane biophysical properties, and to explore their roles in physiological and pathological membrane dynamics.

By bridging the gap between artificial systems and the complexity of living cells, our approach yields valuable insights into lipid-mediated processes at the molecular level, facilitating a deeper understanding of cellular functions.

# Oral Communications

# O1. Development of Metal-based Molecularly Imprinted Polymers in Supercritical CO$_2$ for Biorecognition Applications

Furtado, Ana I. (1,2); Bonifácio, Vasco D. B. (2); Viveiros, Raquel (1); Casimiro, Teresa (1)

(1) CleanMIPTech group, LAQV-REQUIMTE, Chemistry Department, NOVA School of Science & Technology, NOVA University of Lisbon, Portugal
(2) iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon, Portugal
E-mail: ai.furtado@campus.fct.unl.pt

The Periodic Table has an extensive collection of chemical elements, many of which have not yet been fully explored, providing an opportunity and a challenge to develop new chemistry and materials. Metals are elements that have great potential in the preparation of selective binding sites within polymeric matrices through molecular imprinting, for biorecognition [1]. Molecular Imprinting is a synthetic way to obtain crosslinked polymers with specific affinity sites, complementary in terms of size, conformation, and functionality of the target molecule, known as Molecularly Imprinted polymers (MIPs). MIPs mimic natural molecules in their molecular recognition ability, with significant advantages when compared to antibodies, since are cost-effective, robust, stable under harsh conditions, have a long lifetime, and are easily stored [2]. The production of metal-based MIPs using green technologies, such as supercritical carbon dioxide (scCO$_2$) technology, is still limited and is an opportunity to explore new sustainable routes to produce these materials. CO$_2$ is abundant, non-toxic, non-flammable, with a very accessible critical point and easily tuned solvent power by simply adjusting pressure and temperature which makes its use very attractive as reaction medium [3]. MIP production under scCO2 has proven its potential in the development of high-value materials, inclusive materials for biorecognition applications [4]. Herein, it is described the synthesis of a new class of Metal-functional materials towards an amino acid using scCO$_2$ technology. This technology allowed the preparation of MIPs as dry, ready-to-use, stable, and high-affinity polymeric materials, presenting an imprinting factor (*IF*) of 12, and revealing a powerful tool for developing advanced biorecognition materials for a wide range of applications.

**References:** [1] Song, Z et al. (2022) Molecularly imprinted polymers-based materials and their applications in chromatographic and electrophoretic separations, TrAC, Trends Anal. Chem, 146, 1-19. [2] Z. El-Schich, Z. et al. (2020), Molecularly imprinted polymers in biological applications, BioTechniques, 69, 407-420. [3] Furtado, A.I. et al. (2021) MIP Synthesis and Processing using Supercritical Fluid, Methods mol. biol., (2021) 2359, 19-42. [4] Furtado, A.I. et al. (2023) Biomolecular Fishing: Design, Green Synthesis, and Performance of L-Leucine-Molecularly Imprinted Polymers, ACS Omega, 8, 9179-9186.

# O2. Inhibition of Aβ42 oligomers relevant in Alzheimer's disease by a chaperone multimer

Figueira, António J. (1,2); Gomes, Cláudio M. (1,2)

(1) BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
E-mail: ajfigueira@fc.ul.pt

Aggregation of the amyloid-β 1-42 peptide (Aβ42) into fibrils and toxic oligomers is a major hallmark of Alzheimer's disease (AD) and understanding how chaperones harness such conformers is critical to inspire mechanism-based therapies. This includes S100B, an AD up-regulated alarmin that occurs in the brain mostly as a dimer and tetramer [1] and whose Aβ42 anti-aggregation activity was recently unveiled [2, 3]. S100B multimers interact effectively with fibrillar Aβ, suggesting an impairment of Aβ42 nucleation catalyzed by fibril surfaces. This auto-catalytic mechanism primes the massive generation of Aβ42 oligomers (AβO) [4], which prompted us to hypothesize that S100B could regulate the formation of such neurotoxic species. To investigate this possibility, we first resorted to thioflavin-T monitored Aβ42 aggregation assays coupled to mechanistic analysis, which provides information about the microscopic mechanisms governing fibrillation. Complying with the targeting of fibrils, mechanistic analysis revealed that both dimeric and tetrameric S100B – the latter operating under sub-stoichiometric conditions – inhibit preferentially Aβ42 surface-catalyzed nucleation. Through simulations of AβO temporal distributions, we found that S100B multimers decrease Aβ42 oligomerization rate by ~90% and minimize the total amounts of AβO formed. To verify this mechanism-derived prediction, we also established a fluorescence-based approach to independently evaluate the formation of AβO using a combination of thioflavin-T and X-34, a Congo red derivative which we show is able to detect early thioflavin-negative Aβ42 conformers. Coincidently, we observed an S100B-mediated depletion of X-34 positive AβO that recapitulates mechanistic outcomes. Altogether, our study sheds new insights on the catalytic landscape of the S100B chaperone, suggesting its critical role in the regulation of protein aggregation and oligomer formation in AD [5].

**References:** [1] Ostendorp T. et al (2007) Structural and functional insights into RAGE activation by multimeric S100B. EMBO J., 26, 3868–3878; [2] Cristóvão J.S. et al (2018) The neuronal S100B protein is a calcium-tuned suppressor of amyloid-β aggregation. Sci. Adv., 4, 1702; [3] Figueira A.J. et al (2022) Tetramerization of the S100B Chaperone Spawns a $Ca^{2+}$ Independent Regulatory Surface that Enhances Anti-aggregation Activity and Client Specificity J. Mol. Biol., 434, 167791; [4] Michaels, T.C.T. et al (2020) Dynamics of oligomer populations formed during the aggregation of Alzheimer's Aβ42 peptide Nat. Chem. 12, 445–451; [5] Figueira A.J. et al (2023) S100B chaperone multimers suppress the formation of oligomers during Aβ42 aggregation Front. Neurosci., 17, 1162741.

# O3. Describing halogen anisotropy in solvation, membrane permeability, and protein-ligand systems: towards better drugs

<u>Fortuna, Andreia (1,2)</u>; Costa, Paulo J. (1)

(1) BioISI - Instituto de Biossistemas e Ciências Integrativas and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
(2) iMed.ULisboa - Research Institute for Medicines, Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal
E-mail: ajfortuna@fc.ul.pt

Halogenation is a widely employed strategy in drug discovery aimed at optimizing absorption, distribution, metabolism, and excretion properties. Additionally, halogenated compounds have the ability to interact with biological targets, including proteins [1] and membranes [2], through the formation of halogen bonds (XB). This odd R-X···B interaction, where X is a halogen atom and B a Lewis base, arises from the anisotropic character of the electrostatic potential on X, which possess a positive site on its type, enabling XBs, while a negative belt is found around the halogen atom which allow X to act also as a hydrogen bond (HB) acceptor.

Describing anisotropy is challenging using force field methods that rely on atomic charges (e.g. X atoms are negative, therefore, XBs are not possible). To tackle this, our group has been developing and testing parameters aiming at improving the description of halogens in important biological phenomena such as solvation, receptor binding, and membrane permeability. In this context, the role of odd noncovalent interactions such as XBs is still largely overlooked since the analysis is usually focused on HBs. Herein, we will show these recent advances namely, the study and validation of different halogen parameters to calculate hydration free energies ($\Delta G_{hyd}$) using implicit (fast) [3] and explicit (more detailed) solvent models. Moreover, since the desolvation process is important in protein-ligand and membrane-ligand interactions, these parameters were also studied in the determination of binding free energies ($\Delta G_{bind}$) and calculated permeability ($P_{calc}$) of halogenated compounds.

**References:** [1] Costa, Paulo J. et al. (2023). Halogen bonding in halocarbon-protein complexes and computational tools for rational drug design. Exp. Expert Opin Drug Discov. 14, 805-820; [2] Nunes, Rafael, et al (2021). Halogen Bonding: An Underestimated Player in Membrane−Ligand Interactions. J. Am. Chem. Soc. 11, 4253−4267; [3] Fortuna, Andreia et al. (2021). J. Chem. Inf. Model. Optimized Halogen Atomic Radii for PBSA Calculations Using Off-Center Point Charges. J. Chem. Inf. Model. 7, 3361-3375.

# O4. Proteomic profiling of astroglial and microglial cell models of the Autosomal Recessive Ataxia of Charlevoix-Saguenay (ARSACS)

Murtinheira, Fernanda (1,2); Boasinha, Ana (1); Macedo, Luana (1); Nascimento, Patrícia (1); Pinto, Francisco (1); Torres, Vukosava (1); Rodrigues, Mário (1); Herrera, Federico (1)

(1) BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências da Universidade de Lisboa, 1749-016, Lisbon, Portugal
(2) ITQB NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157, Oeiras, Portugal
E-mail: fiferreira@fc.ul.pt ; fherrera@fc.ul.pt

Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS) is the second most prevalent form of ataxia worldwide and occurs due to mutations in the *SACS* gene leading to low expression or dysfunction of the sacsin protein. Sacsin is a very large protein (520 kDa) highly expressed in the central nervous system, including astrocytes and microglia, but only its neuronal role has attracted attention. Our research aimed to investigate the impact of sacsin deficiency in glial cells and its association with ARSACS. Sacsin knockout in C6 rat astroglial-like cells and HMC3 human microglial cells disrupted the subcellular distribution of glial intermediate filaments and membrane organelles. It also impaired autophagy and specific stress pathways in the endoplasmic reticulum. $C6^{Sacs-/-}$ cells exhibited faulty STAT3 signalling in response to inflammatory cytokines and increased susceptibility to several forms of stress, such as mitochondrial respiration inhibition, serum deprivation and irradiation. The proteomic profile of $C6^{Sacs-/-}$ cells showed 196 differentially expressed proteins (36 upregulated and 104 downregulated) related to some of these alterations. These findings suggest that sacsin plays a crucial role in glial cells and that astrocytes and microglia may contribute to ARSACS pathogenesis. The $C6^{Sacs-/-}$ astroglial-like and $HMC3^{Sacs-/-}$ microglial cell models could also offer valuable insights into other human disorders associated with disruptions in intermediate filament networks, such as Giant Axonal Neuropathy and Alexander disease. Novel therapeutic strategies targeting glial cells rather than neurons could constitute a unique and original approach to the treatment of ARSACS and similar disorders.

# O5. Innovative smart metallodrug delivery systems for targeted therapy of metastatic breast cancer

Franco Machado, João (1,2); Silva, Miguel (2); Pires, Inês (3); Marques, Fernanda (2); Machuqueiro, Miguel (3); Garcia, M. Helena (1); Correia, João D. G. (2); Morais, Tânia S. (1)

(1) CQE, IMS, DQB, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
(2) C2TN, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
(3) BioISI, DQB, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
E-mail: jmfmachado@ciencias.ulisboa.pt

Metastatic breast cancer (MBC) remains one of the most common and lethal type of tumours worldwide. The median survival time of patients with MBC is merely 2 years, mostly due to the lack of effective drugs capable of reaching metastases and the inadequate selectivity of current treatments towards cancer cells that results in severe adverse effects. [1]

Recently, we have been dedicated to developing an innovative family of smart metallodrug delivery systems capable of targeting with high precision MBC cells, therefore sparing the healthy tissues. These systems consist of novel ruthenium-peptide conjugates (RuPCs) that selectively recognize the fibroblast growth factor receptor (FGFR) overexpressed by MBC cells [2], and controllably release a highly cytotoxic organometallic ruthenium complex upon the stimulus of the acidic tumour microenvironment. Thus, these RuPCs may promote enhanced therapeutic efficacy with reduced side effects.

In this communication, we report the synthesis, structural characterization, and *in silico*/*in vitro* biological evaluation of new RuPCs. The interaction with their molecular target (cell membrane) was studied by molecular dynamics simulations. The drug release profile in aqueous solution was evaluated at pH values that mimic both the tumour microenvironment and the healthy tissues/bloodstream. The cytotoxicity and selectivity were determined in a panel of human breast cancer cells and non-tumoral fibroblasts with different levels of FGFR expression at the referred pH values.
The lead RuPC showed controlled release of the ruthenium complex in its active form allied to selective antiproliferative activity against FGFR(+) MBC cells, suggesting its potential use as a novel agent for the precision therapy of MBC.

**References:** [1] Tufail, M. et al. (2022) Breast cancer: molecular mechanisms of underlying resistance and therapeutic approaches. Am J Cancer Res, 12, 2920-2949; [2] Franco Machado, J et al. (2020) Novel "ruthenium cyclopentadienyl"-peptide conjugate complexes against human FGFR(+) breast cancer. Dalton Trans, 49, 5974-5987.

# O6. Generative Adversarial Networks for untargeted metabolomics data augmentation, friend or foe?

Traquete, Francisco (1); Sousa Silva, Marta (1); Ferreira, António E.N. (1)

(1) Laboratório de FT-ICR e Espectrometria de Massa Estrutural, BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa
E-mail: fmtraquete@fc.ul.pt

Metabolomics is a major analytical tool for the molecular characterization of biological systems and biomarker identification. Nevertheless, metabolomics data analysis faces many challenges. Two such issues are the need for large sample sizes to train neural networks [1] or class imbalance (a minority class has fewer samples available). The latter can make statistical models less generalizable, increasing the risk of underfitting the minority class [2] and skewing the analysis towards the majority classes. Data augmentation is a tool that might mitigate these problems.

In this work, we used Generative Adversarial Networks (GAN) [3], specifically Conditional Wasserstein GANs with Gradient Penalty (CW-GAN-GPs) to generate realistic artificial samples and perform data augmentation. Furthermore, we propose and applied several criteria to evaluate metabolomics artificial data quality. We trained CW-GAN-GP models for a set of benchmark datasets (after data pre-treatment). Then, we compared the characteristics of the generated samples to the experimental samples according to several statistical criteria and the performance of supervised statistical models. We observed that the models created realistic artificial data of specific biological classes with matching characteristics to experimental samples, occupying all sample space and avoiding mode collapse. We demonstrate that a model trained on an imbalanced dataset can be much improved by supplementing the minority class with GAN-based samples, when the dataset classes are well defined. However, in case of considerable class overlap, performance gains can be negligible. We therefore conclude that the benefits of data augmentation depend on the quality of the original data. Although data augmentation improves the performance of machine learning models, its application in metabolomics datasets is still scarce and requires the use of criteria for evaluating the generated data quality.
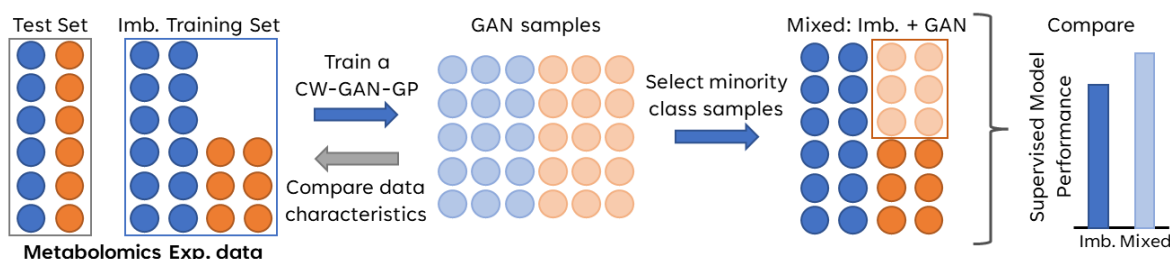


**Fig. 1.** Workflow to generate GAN samples and test their quality and impact (in supervised analysis) compared to experimental datasets. Imb.: Imbalanced; Exp.: Experimental.

**References:** [1] Jiang, X et al. (2020) Generative Adversarial Network Model for Disease Gene Prediction with RNA-Seq Data. IEEE Access, 8, 37352–37360; [2] Zheng, W et al. (2020) The Effects of Class Imbalance and Training Data Size on Classifier Learning: An Empirical Study. SN Comput. Sci., 1, 1-13; [3] Goodfellow, I et al. (2014) Generative Adversarial Networks. Adv. Neural Inf. Process. Syst., 3.

# O7. Synthesis of psychoactive cathinones and its metabolites: the role of metabolism

Lopes, Rita P. (1,2,4); Miranda, Cláudia C. (3,4,5); Gaspar, Helena (2); Antunes, Alexandra M. M. (1)

(1) Centro de Química Estrutural – Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa.
(2) BioISI – BioSystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa.
(3) AccelBio – Collaborative Laboratory to Foster Translation and Drug Discovery, Cantanhede, Portugal.
(4) iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa.
(5) Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa.
E-mail: rita.padinha.lopes@tecnico.ulisboa.pt

The emergence of new psychoactive substances (NPS) in the recreational drug market presents significant risks to public health due to their potential toxicity and poses challenges in forensic and clinical contexts [1]. The rapid influx of new substances into the market complicates the efforts of authorities to develop the necessary analytical methodologies and conduct metabolic studies for identifying parent drugs and their metabolites [2].

Among NPS, synthetic cathinones represent the largest group seized in Europe and the second largest group reported to EMCDDA in terms of the number of substances [3]. These compounds undergo extensive metabolism, and some of their metabolites can serve as biomarkers of consumption, thereby extending the detection window beyond that of the parent drug.

In our proactive response to the forensic and health problems associated with cathinones, we have successfully synthesized and characterized ten cathinone standards and their corresponding reduced metabolites. Currently, all synthesized target compounds are undergoing toxicity evaluation in the SH-SY5Y cell line. Preliminary results indicate that certain reduced metabolites exhibit higher toxicity than the parent drug. Further studies will be conducted to elucidate the underlying mechanism of this toxicity.

**References:** [1] Lopes, R. P. et al. (2023) Metabolic stability and metabolite profiling of emerging synthetic cathinones. Front. Pharmacol, 14, 1-14; [2] Lopes, B. T. et al (2021) Metabolic profile of Four Selected Cathinones in Microsome Incubations: Identification of Phase I and II Metabolites by Liquid Chromatography High Resolution Mass Spectrometry. Front Chem, 8, 1-13; [3] EMCDDA (2022a). European drug report 2022: Trends and developments. Available at: https://www.emcdda.europa.eu/publications/edr/trends-developments/2022_en (Acessed June 5, 2023).

# O8. Into the Early Stages of Protein Aggregation: How Monomers generate Polymers

Vitorino, João (1); Oliveira, Nuno (1); Rodrigues, Filipe (1); Faísca, Patrícia (2); Machuqueiro, Miguel (1)

(1) BioISI: Biosystems and Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) BioISI: Biosystems and Integrative Sciences Institute, Departamento de Física, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
E-mail: jnvitorino@fc.ul.pt

Protein aggregation is a defining trait of many conformational diseases, such as Parkinson's and Alzheimer's disease [1]. These processes can start with an increased propensity of a monomeric species towards self-association, forming dimers, and subsequently promoting the formation of potential polymer-like chains [2,3].

In Dialysis Related Amyloidosis (DRA), aggregation of protein beta-2 microglobulin (B2M), occurs in patients undergoing long-term hemodialysis. This work focuses on the aggregation-prone monomeric state I2 populated by the D76N mutant of B2M [4], s. Monte Carlo Ensemble Docking (MCED) and Molecular Dynamics (MD) simulations were employed to generate and assess the stability and binding affinity of various I2 dimer configurations [3]. An in-house implementation of the Molecular Mechanics Poisson Boltzmann Surface Area Method (PyBindE, available at: https://github.com/mms-fcul/PyBindE) was utilized to calculate binding energies and determine key binding forces. Stable binding interfaces with crucial residues were identified. Clustering protocols and extended MD simulations confirmed the stability and growth potential of select binding modes. Additionally, we explore the plausibility of polymerization, using a novel simple polymer growth model based on a minimal representation of binding interfaces, that is capable of predicting several types of unlimited and limited growth modes.
Our comprehensive framework provides valuable insights into the growth potential and stability of B2M binding interfaces, shedding light on the mechanisms underlying protein aggregation in conformational diseases. Moreover, the universality of the methodologies employed in this study offers a versatile toolset for probing protein dynamics in a broader scientific context [5].

**References:** [1] Chiti, F. et al. (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem. 333–366; [2] Ilie I.M. et al. (2009) Simulation Studies of Amyloidogenic Polypeptides and Their Aggregates. Chem Rev. 119, 6956–6993; [3] Oliveira N.F.B. et al. (2021) Predicting stable binding modes from simulated dimers of the D76N mutant of β 2-microglobulin. Comput Struct Biotechnol J. 19, 5160–5169.[4] Loureiro R.J.S. et al. (2020) The Early Phase of β2-Microglobulin Aggregation: Perspectives From Molecular Simulations. Front Mol Biosci. 7, 578433; [5] Oliveira N.F.B. et al. (2023) Interfacial Dynamics and Growth Modes of β2-Microglobulin Dimers. J Chem Inf Model.

# Flash
# Communications

# F1. Synthesis and Characterization of Copper (I) complexes and study of their catalytic activity in benzyl alcohol oxidation

Machado, Beatriz P. (1); Gomes, Clara S. B. (1); Rosa, Vitor (1)

(1) LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology (FCT-NOVA), NOVA University Lisbon, 2829-516 Caparica, Portugal.
E-mail: bp.machado@campus.fct.unl.pt

The chemoselective and regioselective oxidation of primary alcohols to yield only aldehydes is a reaction of great interest in organic chemistry. Benzyl alcohols and their derivatives are among the most abundant compounds in biomass and can be used as renewable starting materials. To date, their oxidation to aldehydes has typically been performed using toxic, difficult-to-dispose-of compounds and harsh conditions, e.g. Dess-Martin periodinane, Swern reaction, and pyridinium chlorocromate (PCC)[1] .

Following the pioneering work of S. Stahl and co-workers, who used Cu(I) salts, in the presence of bi-pyridine, an organic radical precursor, and a base, to selectively obtain benzaldehyde derivatives using air as a co-oxidant, we focused our efforts on the synthesis of a family of Cu(I) complexes, bearing a diazabutadiene (DAB) or a bis(imino) acenaphthene (BIAN) ligand (Fig. 1), as potential catalysts for the aerobic oxidation reaction of benzyl alcohol. The main difference between these two families of ligands is the steric hindrance in the complex backbone, which leaves the metal center more or less free to react with the substrate. The synthesized compounds allowed us to study the effect of the counter-ion (OTf and $BF_4$) and the effect of the benzonitrile and acetonitrile on the stability of the complex. All the synthesized compounds were fully characterized by spectroscopic methods and, when possible, by X-ray crystallography and were found to be active in the oxidation of benzyl alcohol into aldehyde. Supplementary studies were carried out to investigate the effect of the solvent, substrate and catalyst concentration on the catalyst performance and the reaction substrate scope.



**L1**: $R_1$= Me
**L2**: $R_1$= H
**L3**: $R_1$= acenaphthene

**C1**: $R_1$= Me
**C2**: $R_1$= H
**C3**: $R_1$= acenaphthene

**C4**: $R_1$= Me
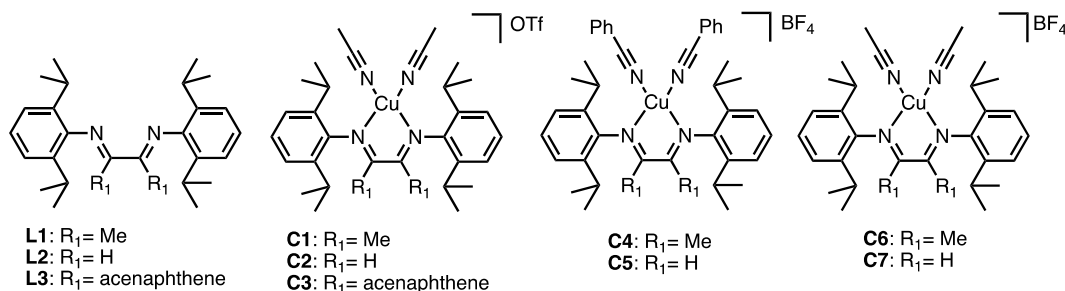**C5**: $R_1$= H

**C6**: $R_1$= Me
**C7**: $R_1$= H

**Fig. 1.** Synthesized ligands and complexes.

**References:** [1] Hoover, J.M. et al. (2012) Copper(I)/tempo-catalyzed aerobic oxidation of primary alcohols to aldehydes with ambient air, Nat Protoc. 7, 1161–1166.

# F2. Unveiling the Functional Role of TGR5 in Fat-to-Liver Communication: Discovery of Novel Agonists

Farinha, B (1,2); Brigido, AM (1), Simão, AL (1), Victor, BL (2), Castro, RE (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal
(2) Biosystems and Integrative Systems Institute (BioISI), Faculty of Sciences, Universidade de Lisboa, Portugal
E-mail: beatriz.farinha@edu.ulisboa.pt

Due to the global obesity epidemic, the prevalence of non-alcoholic fatty liver disease (NAFLD), the main cause of chronic liver disease, has rapidly increased. The pathophysiology of NAFLD involves complex interactions between different organs, including the liver and the adipose tissue (AT). It has been shown that activating AT Takeda G-protein-coupled receptor 5 (TGR5) receptor increases energy expenditure and ameliorates obesity and insulin resistance, while AT-derived exosomal miRNAs were found to regulate mRNA translation in the liver. Through a multidisciplinary approach, combining computational and experimental methods, here we aimed to better understand TGR5 activation in adipocytes, exploring the impact on exosomal content and its functional role in stressed liver cells.

Our approach involved an initial structural characterization of TGR5 and the identification of new agonists through a structure-based virtual screening approach. By docking molecules from the MyriaScreen Diversity Collection compound database [1] to the available TGR5 PDB structures [2,3], we selected promising compounds based on their binding affinity and ability to form hydrogen bonds with Y240, a key residue influencing TGR5's affinity and selectivity. These compounds have been purchased and are being functionally tested *in vitro* in parallel with previously reported and commercially available TGR5 agonists. Additionally, we isolated extracellular vesicles (EVs) from adipocytes and characterized them. We observed that EVs from TGR5-activated adipocytes contained anti-inflammatory molecules capable of reducing the inflammatory response of hepatocytes stimulated with lipopolysaccharide (LPS). Conversely, EVs from TGR5-silenced adipocytes or LPS-stimulated adipocytes triggered an inflammatory response in hepatocytes, leading to increased expression of pro-inflammatory cytokines and enhanced cell death. Overall, our findings suggest that TGR5 activation in adipocytes can modulate EV release and influence the inflammatory response in hepatocytes, highlighting the potential of TGR5 agonists for the treatment of metabolic diseases.

**References:** [1] MyriaScreen Diversity Collection. MyriaScreen II http://myriascreen.com; [2] Yang, F. et al. (2020) Structural basis of GPBAR activation and bile acid recognition. Nature 587, 499–504; [3] Ma, L. et al. (2022) Structural basis and molecular mechanism of biased GPBAR signaling in regulating NSCLC cell growth via YAP activity. Proceedings of the National Academy of Sciences of the United States of America 119.

# F3. Electrocatalytic Ammonia Conversion Using Metal-Organic Frameworks Films

Borralho, Duarte (1); Martinho, Paulo N. (1); Melo Jorge, Maria E. (2); Realista, Sara (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento 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
E-mail: fc51349@alunos.fc.ul.pt

As a measure to fight climate change caused by the excessive use of fossil fuels, $H_2$ as a non-carbon green fuel is a great alternative fuel. However, its implementation is hampered due to properties such as low volumetric energy density, flammability and volatility, leading to storage and transportation challenges. One solution to this problem is using a $H_2$ carrier such as $NH_3$, a non-flammable chemical with a well-known storage technology that can be converted to produce $N_2$ and $H_2$, while having the advantage of a lower conversion potential when compared with $H_2O$ [1].

The focus of this work is the synthesis and immobilisation of metal-organic frameworks (MOFs) on electrodes to be used in the electrocatalytic conversion of $NH_3$ to $H_2$, owing its interest to its properties [2]. Two methods of deposition were performed, a direct method (cathodic deposition) which uses the MOF precursors and an indirect method (electrophoretic deposition) where the MOF is previously synthesised. The films formed (fig. 1. (a)) were characterised by infrared spectroscopy, x-ray diffraction and scanning electron microscopy. $NH_3$ conversion studies using MOF films were performed using cyclic voltammetry (fig. 1. (b)) and controlled potential electrolysis experiments. $H_2$ was quantified using gas chromatography with a thermal conductivity detector.
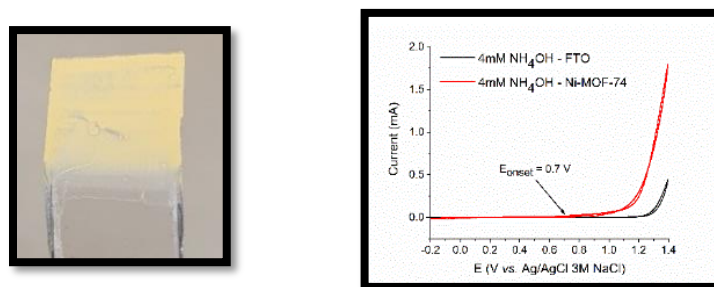


**Fig. 1.** (a) Ni-MOF-74 deposited on FTO. (b) Cyclic voltammetry studies using (a) in presence of $NH_3$.

**References:** [1] Aziz, M. et. al. (2020) Ammonia as Effective Hydrogen Storage: A Review on Production, Storage and Utilization. Energies, 13, 3062; [2] Jiao, L. et. al. (2018) Metal–Organic Frameworks as Platforms for Catalytic Applications. Advanced Materials, 30, 1703663.

# F4. Understanding Metastasis Organotropism Patterns Through Within-cell and Between-cells Molecular Interaction Networks

Miranda, João A. I. (1,2); Pinto, Francisco R. (1,2)

(1) RNA Systems Biology Lab, BioISI
(2) Departamento de Química e Bioquímica
E-mail: jamiranda@ciencias.ulisboa.pt

Metastasis is responsible for the majority of cancer-related deaths. It occurs when cells from a primary tumour disseminate and initiate new tumours at distant organ sites. [1] Metastasizing cells have to exhibit especial characteristics that allow them to surpass all barriers and bottlenecks in their way to effective colonization. Ensuring survival throughout this process depends on how those cells communicate with the surrounding environments. Patterns of metastasis are remarkably variable between cancer types. [2] In fact, distinct cancers seem to be predisposed to metastasize to specific organs, a feature known as metastasis organotropism. [3] Our work is based on the hypothesis that organotropism can be partially explained by the extent of intercellular communication between metastasizing cells and cells in the secondary organ. Some proteins that establish intercellular interactions are tissue-specific and can be expressed in pre-cancerous tissue.

Using RNA-seq data from non-diseased tissue, we built networks of intercellular protein-protein interactions between cells from the primary cancer tissue and cells from a potential metastasis tissue. Controlling for other factors that affect organotropism, we found that sites where cancers metastasize more often tend to establish a larger number of intercellular interactions than sites with low incidence of metastasis. We detected 528 literature curated interactions that might play a role in metastasis formation and contribute to the observed differences in cell-cell communication, some previously known to be related to cancer and/or metastasis. Finally, using a network of signalling pathways, we observed that proteins involved in metastasis-associated interactions and their closest neighbours in the network are enriched in cancer driver genes and biological processes linked to invasion and metastasis. Our findings revealed intercellular interactions and proteins that drive metastasis development and help explain organotropism. These new insights might constitute new research and therapeutic opportunities for treating and preventing metastases.

**References:** [1] Gerstberger, S. et al. (2023) Metastasis. Cell 186, 1564–1579; [2] Lambert, A.W. et al. (2017) Emerging Biological Principles of Metastasis. Cell 168, 670–691; [3] Obenauf, A.C. et. al. (2015) Surviving at a Distance: Organ-Specific Metastasis. Trends in Cancer 1, 76–91.

# F5. Photocatalytic nanocomposite for emerging pollutants remediation

Hassan Ali (1); Olinda Monteiro (2)

(1) Centre of Polymer Systems, Tomas Bata University in Zlin, tr. T. Bati 5678, 760 01 Zlin, Czech Republic
(2) Departamento de Química e Bioquímica, Centro de Química Estrutural – Institute of Molecular Sciences,
Faculdade de Ciências, Universidade de Lisboa
E-mail: hali@utb.cz

In this study, we report on a silica-supported graphitic carbon nitride photocatalyst synthesized via a facile heat treatment route of melamine and characterized by XRD, SEM, and UV-vis spectroscopy. The photocatalytic activity of the as-prepared sample was evaluated by the elimination rate of four major estrogenic hormones (E1, E2, EE2, E3) under visible light irradiation at $\lambda_{max} \sim 425$ nm. The degradation results showed that the as-prepared samples possessed considerable adsorption affinity for all tested hormones as well as robust photocatalytic efficiency. Owing to the synergistic adsorptive and photocatalytic activity, elimination rates of 82, 93, 83, and 96% were achieved for the E3, E2, EE2, and E1 hormones, respectively. In addition, antibacterial tests showed that the as-prepared sample possessed reasonable antibacterial activity against the gram-positive (*Staphylococcus aureus*) and while almost no activity was observed for the gram-negative (*Escherichia coli*) bacteria.
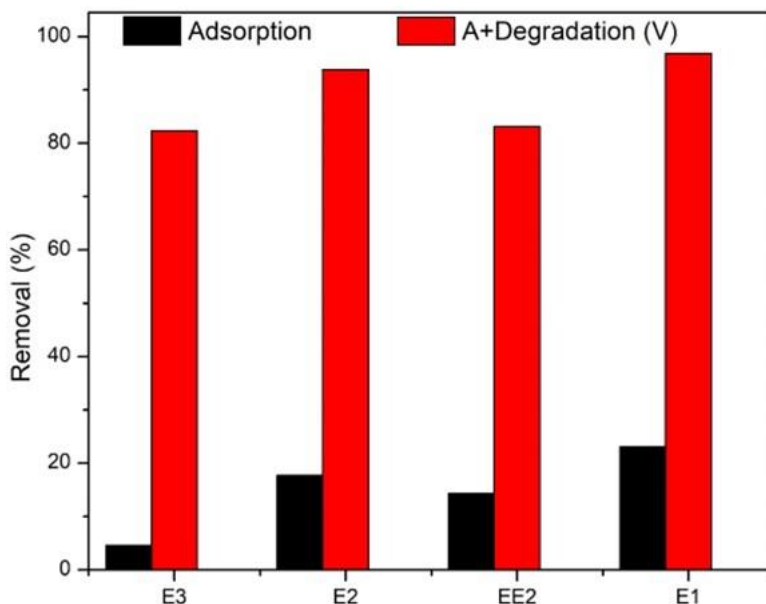


**Fig. 1.** Adsorption and Visible-Photocatalytic degradation of steroid estrogen hormones in 60 min.

# F6. Scalable production of bacteriophages preparations

Saavedra, J.; Duarte, S. (1); Silva-Santos, A.R. (1); Prazeres, D.M.F. (1); Azevedo, A.M. (1)

(1) iBB – Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal
E-mail: joao.saavedra@tecnico.ulisboa.pt

Due to the emergence of antibiotic-resistant bacteria, minor wounds and infections are likely to become potentially fatal. Within this context, phage therapy is emerging as an interesting alternative. Phage therapy resorts to bacteriophages (i.e., viruses that infect bacteria) to combat bacterial infections, taking advantage of phages' specificity and low toxicity. The biomanufacturing processes must address, amongst other aspects, the removal of impurities, particularly endotoxins. Endotoxins, or lipopolysaccharides (LPS), are molecules present in the outer membrane of Gram-negative bacteria. The lipid moiety (lipid A) is a potent activator of macrophages and monocytes, which release tumour necrosis factors, interleukins and platelet activating factors that may ultimately result in septic shock. Consequently, removing endotoxins for parenterally-applied medications is mandatory.

The aim of this work was to develop a scalable method for phage production and particularly to remove key impurities such as host DNA, host proteins and endotoxins. Model phage T4 (a lytic virus that infects *Escherichia coli* K12) was used. The host was grown at 37 °C and 250 rpm in tryptic soy broth (TSB) medium and infection occurred at a multiplicity of infection (MOI) of 0.1. Afterwards, the lysate was collected, centrifuged and sterile-filtered. To improve the performance of the anion-exchange chromatography (AEC) step, bacterial DNA was digested with denarase (2 h, 20 U/mL), after which the lysate was diafiltered (five diafiltration volumes) in tangential filtration flow (TFF) mode. To disrupt endotoxins' aggregates, Tween 20 was used above its critical micellar concentration, followed by its removal with activated charcoal (1 g per 0.23 g Tween). The phage stream was then loaded in an AEC QA monolith. Binding and elution were optimised in terms of ionic strength. Adsorption was found to best perform in a 20 mM Tris-HCl buffer at pH=7.5 and 300 mM NaCl. Phage titre, endotoxins, DNA and protein content were evaluated, using respectively the double-agar plaque assay method, the limulus amoebocyte lysate (LAL) test, real-time PCR (RT-PCR) and the bicinchoninic acid assay (BCA) test. A global phage yield was calculated as 19%, with a final concentration of $1.12 \times 10^{12}$ PFU/mL, and high removal of DNA and proteins. The process, however, still needs to be further studied and optimised, so as to guarantee an effective removal of endotoxins from bacteriophages preparations.

# F7. Biomass-derived activated carbons for point-of-use water treatment

Costa, Henrique (1); Mesquita, Elsa (2); Viegas, Rui M.C. (2), Duque, Leidy Peña (3); Andrade, Marta (1); Carvalho, Ana P. (1); Rosa, Maria João (2); Mestre, Ana S. (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
(2) Urban Water Unit, Hydraulics and Environment Department, LNEC-National Laboratory for Civil Engineering, Av. Brasil 101, 1700-066 Lisboa, Portugal.
(3) Millipore Sigma, Bellefonte, Pennsylvania, USA. Current affiliation: Ingevity, North Charleston, SC, USA
E-mail: fc49741@alunos.fc.ul.pt

Proper water quality is essential for human health and several industrial and laboratorial procedures. Point-of-use (POU) water filters have been increasingly adopted by final consumers to improve tap water quality (e.g., removal of color, taste & odor compounds or organic microcontaminants), particularly when they are served by old or compromised water distribution systems.[1] POU devices hold a market value of around 30 billion dollars annually and are estimated to have an annual growth of 7.5% in 2023. Aiming to develop improved POU devices, tailored granular activated carbons (GACs) prepared from pine nut shell (PNS) [2] are being tested using batch adsorption tests (kinetic and isotherms) and rapid small scale column tests (RSSCTs)[3] to assess their efficiency on dissolved organic matter (DOM, measured as total organic carbon and as absorbance at 254 nm) and residual chlorine removal from tap water. PNS/GACs were prepared by steam or $CO_2$ activation and sieved to obtain fractions with adequate particle size distribution for the RSSCTs and POU filters. PNS/GACs surface chemistry properties were assessed, and each fraction was characterized regarding the nanoporous structure. The lab-made materials were benchmarked with a commercial golden standard of mineral origin (F400, CalgonCarbon). All lab-made GACs were significantly more alkaline than the mild acidic F400 (>9.5 vs 5.8). Porosity was also larger when compared to F400 (BET area 1309-1706 $m^2/g$ vs 1314 $m^2/g$). So far, batch tests results show that PNS/GACs outperform commercial F400 for DOM adsorption, with the steam activated PNS/GACs attaining the higher removal efficiencies.
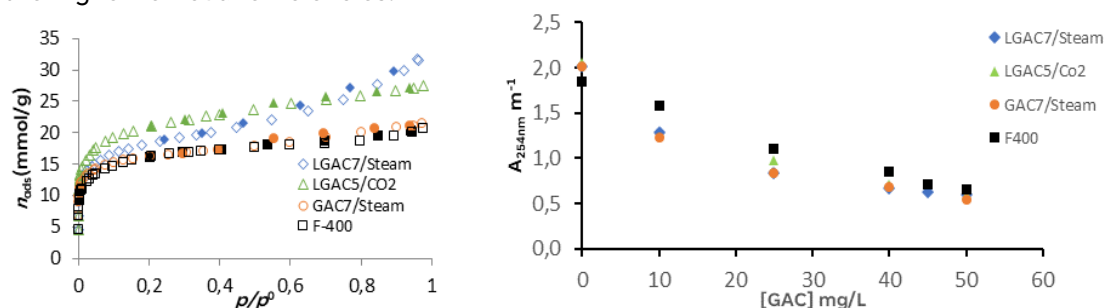


**Fig. 1.** $N_2$ adsorption isotherms (left) and DOM (as $A_{254}$) batch tests results (right).

**References**: [1] WHO (2022), Guidelines for drinking water quality, ed.4th, 614 [2] Mestre, A.S. et. al. (2022) Engineered pine nut shell derived activated carbons for improved removal of recalcitrant pharmaceuticals in urban wastewater treatment J. Hazard. Mater., 437, 129319. [3] Crittenden, J. C. et al. (1991), Predicting GAC Performance with Small Scale Test Column, J AMER WATER WORK ASSN 83, 77-87

# F8. The role of RIPK3 in the crosstalk between hepatocytes and macrophages in experimental models of NAFLD

Alves, Mariana I (1); Afonso, Marta B (1); Rodrigues, Cecília M P (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal
E-mail: falves.mariana@gmail.com

The receptor-interacting protein kinase 3 (RIPK3) is crucial in necroptosis, an immunogenic form of regulated cell death activated in non-alcoholic fatty liver disease (NAFLD). Still, RIPK3 plays controversial roles in the pathogenesis of NAFLD, together with unclear cell-specific functions. Here, we aim to characterize the role of RIPK3 in the crosstalk between hepatocytes and macrophages following fat overload.

Wild-type (WT) and CRISPR-Cas9 Ripk3-null ($Ripk3^{-/-}$) AML12 murine hepatocytes were incubated with 125 µM of palmitic acid (PA) bound to bovine serum albumin for 24 h, followed by co-culture with J774A.1 murine macrophages at different ratios without further treatment. Specifically, we used a co-culture system consisting of plated hepatocytes and macrophages from physiologic 1:8 and 1:4 ratios to the inflammation 1:2 ratio in parallel with monocultures of hepatocytes and macrophages in the presence or absence of PA and lipopolysaccharide (LPS, 1 µg/mL) plus interferon gamma (IFN-γ, 100 U/mL), respectively. After 24 h, cell death was evaluated by the luminescent detection of adenylate kinase release, while the expression of inflammatory markers was assessed by qPCR.

Our results showed that cell death increased ~6-fold in WT hepatocytes treated with PA compared to control. Cell death was significantly reduced in $Ripk3^{-/-}$ hepatocytes. Similar results were obtained in hepatocytes and macrophages co-cultures.

The expression of pro-inflammatory genes *Tnf-α*, *Inos*, and *Nlrp3* increased in J774A.1 macrophages following LPS plus IFN-γ treatment. Similarly, expression of pro-inflammatory markers increased in a macrophage ratio-dependent manner in co-cultures with PA-treated WT hepatocytes, a correlation that was lost when using $Ripk3^{-/-}$ hepatocytes. Indeed, WT macrophages in co-culture with PA-treated $Ripk3^{-/-}$ hepatocytes displayed a less inflammatory profile compared to WT hepatocytes, as evidenced by a decrease of pro-inflammatory markers *Tnf-α*, *Inos*, *Nlrp3*, *Il1β*, and *Il8* mRNA levels.

Overall, our results suggest that targeting RIPK3 in hepatocytes may hinder inflammation upon free fatty acids overload, possibly by inhibiting the immunogenic necroptotic cell death routine that holds the potential to activate macrophages. Future work will dissect the specific role of RIPK3 in macrophage immunophenotype.

# F9. Analysis of biogenic volatile organic compounds in Mediterranean shrubs using headspace-bar adsorptive microextraction (HS-BAµE)

Cerqueira, Jéssica (1); Neng, Nuno (1); Nogueira, José (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
E-mail: fc53052@alunos.fc.ul.pt

Currently, one of the major environmental concerns is the frequent occurrence of forest fires, particularly under extreme atmospheric conditions. Some studies suggest that higher temperatures lead to a greater emission of biogenic volatile organic compounds (BVOCs), produced, and accumulated in different plants, becoming extremely flammable gases in the event of forest fires. Consequently, in the presence of an ignition source, BVOCs can contribute to the spread of forest fires, leading to catastrophic events, as was the case with the 'Pedrogão Grande' tragedy (Portugal) in 2017. [1] Thus, it becomes relevant to study the detailed composition of BVOCs even more in depth, especially the terpenoid fraction, consisting of compounds such as α and β-pinene, limonene, 1,8-cineole, and thymol, since they are among the most abundant monoterpenes in trees and shrubs, and even some oxygenated sesquiterpenes, such as caryophyllene oxide. [2] Therefore, it is important to develop and apply effective methodologies that allow the identification of the main BVOCs present in trees and shrubs, highlighting the use of analytical tools such as bar adsorptive microextraction in the headspace mode, an easy-to-use and eco technique combined with gas chromatography-mass spectrometry (HS-BAµE/GC-MS). [3]

The present work aims to apply, optimize, and validate the HS-BAµE/GC-MS methodology to monitor the main BVOCs emitted from several common shrubs in Portugal, namely *Cistus ladanifer L.* and *Cistus monspeliensis L.*, *Erica scoparia L.*, *Lavandula stoechas L.* and *Thymus villosus L.*. The performance, advantages and limitations of this novel approach is also addressed and compared with conventional techniques, such as headspace-solid phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME/GC-MS).

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# F10. Exploring Epithelial Mesenchymal-Transition (EMT)-associated factors in Cystic Fibrosis

Torres, Raquel G (1); Amaral, Margarida D (1); Torres, Vukosava M (1); Pankonien, Ines (1)

(1) University of Lisboa, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Lisboa, Portugal
E-mail: raquel.torres.2001@gmail.com ; ipankonien@ciencias.ulisboa.pt ; msamaral@ciencias.ulisboa.pt

Cystic Fibrosis (CF) is caused by variants in the CF transmembrane conductance regulator (CFTR) gene encoding a $Cl^-/HCO_3^-$ channel expressed at the apical plasma membrane (PM) of epithelial cells [1,2]. The most common CF-causing variant, p.Phe508del, leads to CFTR misfolding and thus defective PM traffic [1,2]. The absence of functional CFTR at the PM causes a severe imbalance in ion and water transport affecting mainly the airways and thus being respiratory failure the main cause of morbidity and mortality in CF [1]. In addition to its function as an anion channel, CFTR has been associated to other cellular processes, such as epithelial differentiation/polarization, proliferation, and epithelial-mesenchymal transition (EMT) [3,4]. During EMT cells lose their epithelial properties, including cell-cell junctions, apical-basal polarity, cell shape and cytoskeleton organization while they acquire features of mesenchymal cells such as front-rear polarity and cell individualization. Recently, our group found that CF cells actively undergo partial EMT and that EMT-associated transcription factors (EMTa-TFs), such as YAP1, TEAD4, and TWIST1 are implicated in this process and therefore are potential key factors linking EMT with defective CFTR [4,5]. However, it is still unknown how CFTR dysfunction can drive such major cellular alterations as those occurring in EMT. The current work aims to further explore the link between the EMTa-TFs and dysfunctional CFTR using biochemical, proteomics and bioinformatic approaches. To this end, the expression levels of YAP1, TEAD4 , and TWIST1 were analysed in polarized cystic fibrosis bronchial epithelial (CFBE) cells expressing wt- or p.Phe508del-CFTR. While YAP1 and TWIST1 expression levels were found to be increased in p.Phe508del-CFTR expressing cells versus wt-CFTR cells, TEAD4 expression was found to be expressed significantly lower in p.Phe508del-CFTR cells in comparison to wt-CFTR cells. Furthermore, co-immunoprecipiation (co-IP) assays pulling down TWIST1 in wt- and p.Phe508del-CFTR expressing CFBE cells were successfully performed proven by a significant increase in TWIST1 expression levels in Western Blot. Protein complexes from the TWIST1 co-IPs in wt- and p.Phe508del-CFTR cells are currently being analysed by Mass spectrometry. Preliminary data from wt-CFTR co-IP samples confirm that TWIST1 is indeed linked to other EMT/cancer proteins in these cells. Further analysis is ongoing to identify differences in the TWIST1 networks in wt- and p.Phe508del-CFTR cells.

**References:** [1] Boeck, K et al. (2016) Progress in therapies for cystic fibrosis, Lancet Respir Med, vol. 4, no. 8, pp. 662–674; [2] Riordan, J et al. (1979) Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA, Science, vol. 245, no. 4922, pp. 1066–1073; [3] Amaral, M et al. (2020)What Role Does CFTR Play in Development, Differentiation, Regeneration and Cancer?, Int J Mol Sci, vol. 21, no. 9; [4] Quaresma, M et al. (2020) Mutant CFTR Drives TWIST1 mediated epithelial-mesenchymal transition, Cell Death Dis, vol. 11, no. 10; [5] Quaresma, M et al. (2022) Exploring YAP1-centered networks linking dysfunctional CFTR to epithelial–mesenchymal transition, Life Sci Alliance, vol. 5, no. 9.

# Poster
# Communications

# P1. Molecular Dynamic Simulation Study of the Crystal Nucleation of Niacin from Ethanol Solutions

Baptista, Diogo S. (1); Bernardes, Carlos E. S. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
E-mail: dsbaptista@fc.ul.pt

Crystallization from solution is an ancient method used in several areas to obtain pure solid compounds. Despite this, there is still a lack of knowledge about how exactly this process occurs molecularly. The first step in the crystallization process is nucleation, which can be defined as the aggregation of molecules in solution, leading to a nucleus that can grow into macroscopic crystals. Due to its occurrence at the beginning of crystallization, it defines the properties of the resulting material. Thus, understanding this process is key to controlling the crystal particle size distribution, the crystal habit, and the molecular arrangement (e.g., polymorphism) of precipitated materials [1]. As a result, due to the poor knowledge about this event, various problems in the industry have been reported, namely, in the pharmaceutical industry (e.g., Norvir) [2]. Therefore, understanding the nucleation process of crystals is not only an important fundamental question but also a matter of technological importance.

In this communication, the crystallization process of niacin (Fig. 1) from ethanol will be discussed based on molecular dynamics simulation results. Niacin (vitamin B3) is an active pharmaceutical ingredient used, for example, in the control of cholesterol levels [3]. Experimental data on the solubility of this compound in several solvents are available, and no polymorphism is known for this compound, so that, independently of the experimental (simulated) conditions, the same crystal phase is always obtained [4]. The molecular dynamics simulations were carried out for solution with niacin mole fractions of $0.0065 \leq x_{NIC} \leq 0.0085$, at different temperatures. It was observed that the niacin molecules form structures through hydrogen bonding, but a competition of the solvent through the solute acid and base sites prevents the formation of large aggregates. Thus, crystallization must start from these small structures that initially appear in the solution.
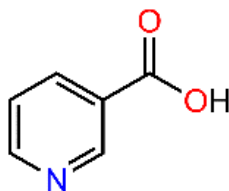


**Fig. 1.** Chemical structure of niacin

**References:** [1] Brittain, H (2009) Polymorphism in Pharmaceutical Solids. 2nd ed.; Informa Healthcare: New York; [2] Bauer, J. et al. (2001) *Pharmaceut. Res.,* 18, 859-866; [3] Gille, A. et al. (2008) *Annu. Rev. Pharmacol. Toxicol.,* 48, 79-106; [4] Gonçalves, E. et al (2012) Solubility of nicotinic acid in water, ethanol, acetone, diethyl ether, acetonitrile and dimethyl sulfoxide J. Chem. Thermodynamics, 47, 362-371

# P2. Study of an alternative methodology to monitor trace levels of Glyphosate in aqueous matrices

Quintelas, Bruno (1); Nogueira, José (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
E-mail: fc56586@alunos.fc.ul.pt

Glyphosate is a broad-spectrum, systemic, non-selective, post-emergent herbicide with the chemical structure shown in Figure 1. Due to its high efficiency and popularity, its use has increased significantly, making it one of the most applied herbicides worldwide [1].

In 2015, glyphosate was classified as "Group 2A – probably carcinogenic to humans" by the International Agency for Research on Cancer. This classification has led to a rapid increase in public concerns regarding the environmental and health risks associated with this substance, particularly due to reports of trace levels of glyphosate in several environmental matrices [2]. Considering the current wide application of this herbicide, several methodologies have been proposed for monitoring trace levels of glyphosate in aqueous matrices [2]. Nevertheless, some of the methodologies used are not fast or are difficult to implement.

In this sense, the present work proposes an alternative methodology for monitoring trace levels of glyphosate in aqueous matrices, using adsorptive microextraction with in-situ derivatization (FMOC-Cl agent), followed by high performance liquid chromatography-diode array detection (BAµE/HPLC-DAD) analysis. In preliminary tests carried out, several sorbents, concentrations of FMOC-Cl and solvents for back extraction were tested, demonstrating to be an alternative methodology in comparison to the well-established ones.
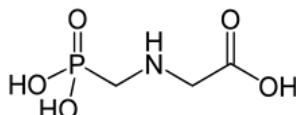


**Fig. 1.** Chemical structure of glyphosate

**References:** [1] Bressán, I. G. et al. (2021) Optimization and Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of Glyphosate in Human Urine after Pre-Column Derivatization with 9-Fluorenylmethoxycarbonyl Chloride. J. of Chrom. B. 1171, 122616; [2] Wang, Shu et al. (2016) A Simple Method for the Determination of Glyphosate and Aminomethylphosphonic Acid in Seawater Matrix with High Performance Liquid Chromatography and Fluorescence Detection. Talanta, 161, 700–706.

# P3. In-depth analysis of solvent effects via aqueous binary mixtures kinetic data

Pinção, Ana (1,2); Moreira, Luís (1,3); Elvas-Leitão, Ruben (1,4); Martins, Filomena (1,2)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
(2) Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa.
(3) Instituto Superior de Educação e Ciências (ISEC Lisboa).
(4) Departamento de Engenharia Química, Instituto Superior de Engenharia de Lisboa, IPL.
E-mail: fc58482@alunos.ciencias.ulisboa.pt

Solvent effects in the reactivity of 2-Cl-2-methylpropane (*t*-BuCl) have been studied using neat media. Some years ago, a successful similarity model was established between rate constants (in terms of -log $k$) and Reichardt's polarity scale ($E_T(30)$): -log $k_{t\text{-BuCl}}$ = - 0.34 $E_T(30)$ + 23.8 ($R^2$ = 0.96), showing that the dipolarity and the HBD acidity of the solvent, both measured by $E_T(30)$, play a significant role in the kinetic process. [1] Solvation processes in pure solvents are intricate and often complex. Using solvent mixtures, increase considerably the degree of complexity, but make the systems much more interesting. Specifically, investigation of aqueous binary mixtures has been a long-standing topic, with extensive data being collected for various solvent properties, among which $E_T(30)$. Computed excess $E_T(30)$ values - $\Delta E_T(30)$ - revealed a non-ideal behavior which was worth exploring. [2] Therefore, in this work we investigated the heterolysis reaction of *t*-BuCl in various aqueous binary mixtures at 25 °C to determine rate constants and the respective excess properties (-log $k^E$). Results were mathematically modeled using Redlich-Kister polynomials [3] and, through interpolation, -log $k^E$ values were compared with the available $\Delta E_T(30)$ values. [2] Our findings show an interesting agreement with the referred similarity model (Fig. 1). However, MeOH-aqueous mixtures exhibit a peculiar behavior which is herein analyzed and discussed.
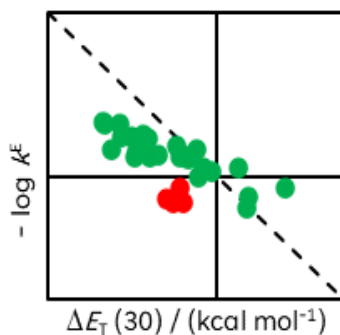


**Fig. 1.** -log $k^E$ *vs*. $\Delta E_T(30)$ in organic-water mixtures at 25 °C: ● MeOH; ● All other cosolvents.

**References:** [1] Bentley, T et al. (2006), Correlations and predictions of solvent effects on reactivity: some limitations of multi-parameter equations and comparisons with similarity models based on one solvent parameter. J. Phys. Org. Chem., 19, 341-349; [2] Marcus, Y (2002) Solvent Mixtures: Properties and Selective Solvation. CRC Press; [3] Redlich, O et al. (1948) Algebraic representation of thermodynamic properties and the classification of solutions. Ind. Eng. Chem., 40, 345-348.

# P4. Surface wettability and its relationship to biocide immobilization in polymeric coatings

Raso, Beatriz (1,2); Santos C.S., M. Soledade (2); Silva R., Elisabete (1)

(1) BioISI-Biosystems & Integrative Sciences Institute, DQB, FCUL, UL, Lisboa, Portugal;
(2) CQE-IMS, DQB, FCUL, UL, Lisboa, Portugal
E-mail: fc56594@alunos.ciencias.ulisboa.pt

Biofouling on submerged surfaces is of global concern, particularly for water distribution and treatment and marine activities (e.g., shipping, Offshore structures). It is associated to biocorrosion of materials and biocontamination of fluid media, representing a critical risk to the sustainability of industrial systems and public health. The biocidal antifouling coating is one of the main strategies to control it [1]. However, biocide blending in coating formulations affects their surface wettability making its assessment crucial for the design and suitability of coatings for application. In this study, the dynamic wettability of developed biocidal polyurethane (PU) and foul-release silicone (Sil)-based coatings [1, 2] in water was evaluated and related to their physicochemical properties. The wettability of the commercial foul-release Sil coatings changed from hydrophobic to hydrophilic behaviour, expressed with dynamic contact angles higher and lower than 90°, respectively. This foul-release behaviour [2] persisted after Econea® biocide blending (0.56 wt.%), but their hydrophilicity decreased, which was associated with the inherent hydrophobicity of the blended biocide. Surface roughness increases have shown a similar trend. In contrast, PU coatings underwent minimal changes, indicating higher system stability upon biocides incorporation.
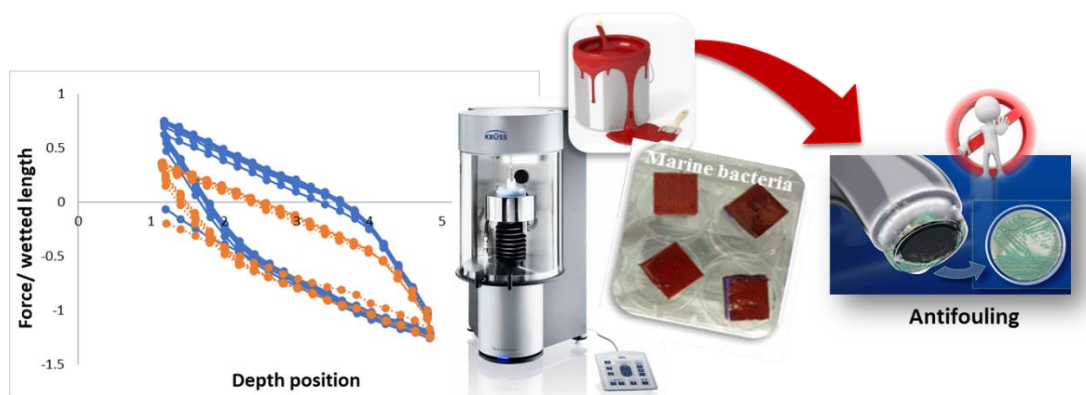


**Fig. 1.** Antifouling coatings' dynamic wettability assessment for design optimization.

**References:** [1] Silva, E.R. et al. (2019) Eco-friendly non-biocide-release coatings for marine biofouling prevention. Sci. Total Environ. 650, 2499-2511; [2] Silva, E.R. et al. (2021) Assessment of the environmental compatibility and antifouling performance of an innovative biocidal and foul-release multifunctional marine coatings. Environ. Res., 198, 111219.

# P5.New ruthenium-antibiotic conjugates for synergistic anticancer/antibiotic activity

Marques, Bárbara (1); Franco Machado, João (1,2); Silva, Miguel (2); Marques, Fernanda (2); Garcia, M. Helena (1); Coelho, Jaime A. S. (1); Morais, Tânia S. (1)

(1) Centro de Química Estrutural – Institute of Molecular Sciences, Universidade de Lisboa, Portugal
(2) Centro de Ciências e Tecnologias Nucleares, IST, Universidade de Lisboa, Portugal
E-mail: tsmorais@fc.ul.pt

Cancer is a leading cause of death, with high morbidity rates and strong economic impact worldwide. Despite cancer aetiology being diverse, several tumours are induced by bacterial infections. Each tumour has also a microbiome that influences its progression, metastasis, and drug sensitivity. Moreover, many patients suffer from opportunistic infections caused by lowered immunity induced by cancer itself and conventional chemotherapy. Most of clinically available treatments still show low efficacy and high adverse effects, impairing a long-term solution for patients with cancer, which urgently claims for novel therapeutic approaches. [1]

Aiming at developing a novel dual-action therapeutic approach, herein we conjugated one of our Ru(II)($\eta^5$-$C_5H_5$) complexes, which already proved to be efficient *in vitro* and *in vivo* against several types of cancer [2], to different antibiotics, to obtain synergistic effects between them and/or modulate the anticancer/antibiotic properties. Thus, we report the synthesis and characterization (NMR, FT-IR, UV-visible spectroscopies) of two new ruthenium-antibiotic conjugates containing a linker sensitive to tumour microenvironment for controlled release of each component (cytotoxic complex/antibiotic), that can potentially be used as a multifunctional anticancer agent (Fig. 1). Their isomeric structure was assessed by density functional theory calculations and their stability in aqueous/organic solutions was determined over time by UV-vis and NMR spectroscopies. The in vitro cytotoxic activity was evaluated in breast (MDA-MB-231), prostate (PC3) and ovarian (OVCAR3) cancer cell lines and will also be discussed.
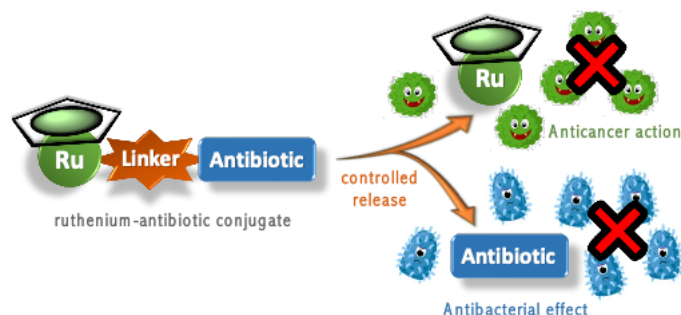


**Fig. 1.** Schematic representation and action of the bifunctional ruthenium-antibiotic conjugates.

**References:** [1] Sung, H et al. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin., 71, 209-249; [2] Morais, T. S. et al. (2016) Tracking antitumor metallodrugs: Promising agents with the Ru(II)- and Fe(II)-cyclopentadienyl scaffolds. Future Med. Chem., 8, 527-544.

# P6. Influence of sphingolipid hydroxylation on membrane organization

Anão, Sofia (1); Bento-Oliveira, Andreia (1); de Almeida, Rodrigo (1); Trigo Marquês, Joaquim (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
E-mail: fc56608@alunos.fc.ul.pt

Fungal infections and their resistance to multiple drugs, considering the few efficient alternatives to date, represent a serious public health problem. [1] Thus, the need arises to investigate and understand the principles underlining the structure and functional organization of fungal plasma membrane, which is the target of many antifungal agents. An important trait of fungal plasma membranes is the presence of sphingolipid-enriched domains (SLEDs), which are in a gel phase under physiological conditions and are not found in mammalian cell membranes. These may constitute a preferential unexplored drug target to overcome the threat of antifungal drug resistance. A key structural difference between fungal and mammalian sphingolipids is the degree of backbone hydroxylation, which is much higher in fungi. Thus, the present work aims to understand the influence of the hydroxylation patterns of sphingolipid on membrane organization, namely in the formation of domains in the gel phase.

To address the main goal of this work, giant unilamellar vesicles (GUVs) prepared by electroformation and examined by confocal fluorescence microscopy were used as membrane model systems. The GUVs contained ceramides (the backbone of complex sphingolipids) differing in their hydroxylation pattern mixed, at 20 and 30 mol % with palmitoyloleoylphosphatidylcholine (POPC) and Rhod-DOPE, a fluorescent probe that labels preferentially fluid domains.

The formation of gel-phase domains could be observed as non-fluorescent regions in all the binary systems studied. Moreover, the influence of ceramide hydroxylation on gel-phase domains became evident as these domains presented different shapes, sizes and abundance clearly depending on the hydroxylation pattern of the ceramide used.

**References**: [1] Vitiello, A. et al. (2023). Antifungal Drug Resistance: An Emergent Health Threat. Biomedicines, 11, 1063.

# P7. Towards luminescent Ru(II)-polypyridyl-biotin complexes as phototoxic agents for cancer treatment

Teixeira, Ricardo G. (1); Tahhan, Bashar (2); Fontrodona, Xavier (3); Romero, Isabel (3); Garcia, Maria Helena (1), Valente, Andreia (1); Romero-Canelón, Isolda (2); Tomaz, Ana Isabel (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.
(2) School of Pharmacy, Institute of Clinical Sciences, University of Birmingham, Edgbaston, United Kingdom.
(3) Departament de Química and Serveis Tècnics de Recerca, Universitat de Girona, C/ M. Aurèlia Campmany, 69, Girona, Spain.
E-mail: rjteixeira@ciencias.ulisboa.pt

Cancer remains a global health concern due to its high mortality rates. While conventional cancer treatments (such as surgery, chemo- or radiotherapy) have been widely utilized, researchers have also explored non-invasive therapies that provide improved precision and fewer side effects. One notable avant-garde approach alternative to chemotherapy is photodynamic therapy (PDT) [1] that consists on using a prodrug in combination with specific light wavelengths. In PDT, the formation of reactive oxygen species (ROS) from cellular oxygen leads to serious oxidative damage of the cells, resulting in targeted cell death.

A strategy to increase treatment selectivity relies on the incorporation of specific biomolecules whose receptors are overexpressed in cancer cells into the photosensitizer (PS) [2]. In the present study, biotin (vitamin B7) has been covalently conjugated with several PS scaffolds. The synthesis, structural and photophysical characterization of a new series of Ru-polypyridyl conjugates will be presented, including investigations into the complexes stability in biologically relevant matrixes, as well as, preliminary cell-based studies on human lung cancer A549 cells exploring their potential as anti cancer agents with irradiations at 460 nm.



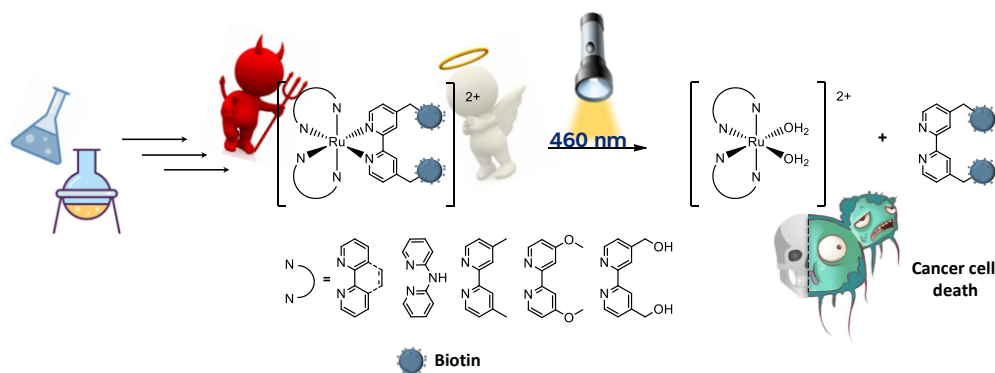**Fig. 1.** Structure-activity relationship study on Ru(II)-polypyridyl complexes incorporating biotin.

**References:** [1] McFarland, S.A. et al (2020) Metal-based photosensitizers for photodynamic therapy: the future of multimodal oncology?. Curr. Opin. Chem. Biol, 56, 23-27; [2] Martínez-Alonso, M. et al (2021) Ruthenium polypyridyl complex-containing bioconjugates. Coord. Chem. Rev., 434, 213736.

# P8. Application of bar adsorptive microextraction to monitor levels of verapamil in plasma samples

Silva, Camilla F. (1,2); Borges, Keyller B. (1); Nogueira, José (2)

(1) Departamento de Ciências Naturais, Universidade Federal de São João del-Rei, Campus Dom Bosco, Praça Dom Helvécio 74, Fábricas, 36301-160, São João del-Rei, Minas Gerais, Brazil.
(2) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail: camillafonsecasilva@aluno.ufsj.edu.br

Verapamil (VER) is an L-type calcium channel-blocking drug, commonly used to treat cardiovascular diseases such as high blood pressure, angina, and arrhythmias. Its therapeutic effects depend on maintaining its plasma concentrations within a safe and effective range (typically, between 20 and 500 ng mL$^{-1}$), while adverse effects are observed at concentrations higher than 1,000 ng mL$^{-1}$. Consequently, efficient therapeutic monitoring of this drug is convenient to achieve the desired clinical benefit and avoid adverse side effects [1,2]. In this regard, a novel analytical approach is proposed to monitor of VER in plasma samples, using bar adsorptive microextraction coated with reversed phase polymers followed by high performance liquid chromatography with diode array detection (BAμE/HPLC-DAD). The chromatographic conditions were: Inertsil ODS-3 column (150 mm × 4.60 mm, 5 μm particle size; GL Sciences) thermostated at 20 °C, and a mobile phase consisted of 0.1% formic acid in water (v/v; phase A) and methanol (phase B), using the following gradient: 0 - 1.5 min: 90% A, 1.5 - 8.0 min: 60% B and 8.0 - 18.0 min: 90% A. The flow rate was 0.6 mL min$^{-1}$, injection volume was 30 μL and detection at 280 nm. Under optimized experimental conditions [microextraction stage - sorbent phase: C18/Ciano polymers, pH sample: 8.0/10.0, equilibrium time: 2 h, stirring speed: 990 rpm; back-extraction stage - methanol: acetonitrile (1:1 v/v), 30 min, under sonication] (Fig. 1), the analytical methodology showed recoveries around 100%, linearity from 20 to 600 ng mL$^{-1}$ ($r^2 \geq 0.99$), in addition to adequate precision and accuracy. Finally, the methodology was applied to real plasma matrices showing to be a promising alternative to monitor trace levels of VER.
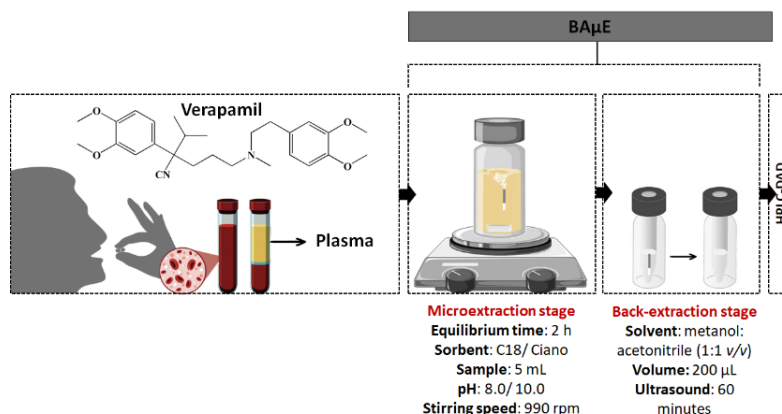


**Fig. 1.** Schematic representation of the optimized methodology.

**References:** [1] Jouyban, A. et al. (2015) Talanta, 134, 681–689. [2] Hefnawy, M. et al. (2021) J. Pharm. Biomed. Anal. 201, 114108-114118.

# P9. Synthesis and pharmacological activity of novel bisquinolizidine derivatives

Ferreira, Daniela R. (1,2); Durão, Raquel M. (1); Fernandes, Ana Margarida M. M. (1); Afonso, Carlos A. M. (1); Coelho, Jaime A. S. (2)

(1) Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa.
(2) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
E-mail: ferreira-daniela@edu.ulisboa.pt

Bisquinolizidine alkaloids, such as (−)-sparteine and (+)-lupanine, are found in several plants of the subfamily Faboideae including the genus Lupinus. These molecules are characterized by a common chiral bispidine core [1] and possess a variety of biological activities, (−) sparteine has both antiarrhythmic [2,3] and anticonvulsant properties and (+)-lupanine is moderately toxic [4]. Our group have been developing methods for the sustainable isolation of these alkaloids [5]. Currently, our research interests include using methodologies for the functionalization of bisquinolizidine alkaloids for medicinal chemistry applications. In this work, we present two synthetic strategies: a) synthesis of 17-substituted lupanine derivatives over the nucleophilic addition of Grignard reagents to the iminium ion derived from lupanine (Figure 1a); and b) synthesis of ammonium salts through N-alkylation reactions (Figure 1b). Finally, we present preliminary results of the biological activity of these bisquinolizidine derivatives.



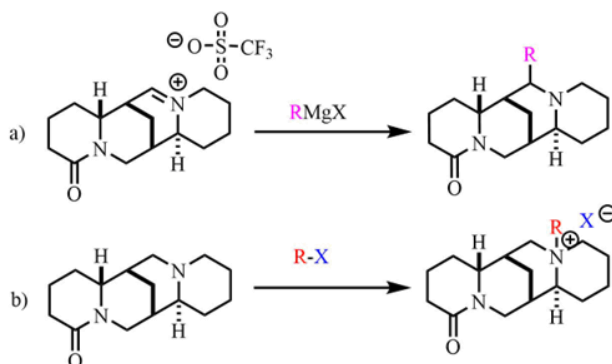**Fig. 1.** Reaction scheme of the addition of Grignard reagents (a) and alkylation reactions (b).

**References:** [1] Goller, J et al. (2019) European J. Org. Chem., 5, 895-9; [2] Senges, J et al. (1973) Arch. Pharmacol, 280, 265-74; [3] Villalpando-Vargas, F et al. (2016) Seizure, 39, 49-55; [4] Scharnagel, D et al. (2018) Angew. Chem., 57, 2456-60; [5] Maulide, N et al. (2014) WO2014/191261.

# P10. New RuCp complex containing a monofunctional 2,2'-bipyridine for metastatic breast cancer

Carvalho, Daniel (1); Piedade, M. Fátima M. (2); Garcia, M. Helena (1); Coelho, Jaime A. S. (1); Morais, Tânia S. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
(2) Centro de Química Estrutural - Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa.
E-mail: fc56597@alunos.fc.ul.pt

Cancer is the second cause of death worldwide, with 19.3 million of new cases and 10.0 million of deaths worldwide only in 2020. Breast Cancer is currently the most common and lethal type of cancer in woman with 4 new cases and 1 death by BC every single minute [1].

The main goal of current cancer therapies is to selectively kill tumour cells while avoiding any damage to healthy cells and tissues. Since this has been the limiting step of any therapy, chemotherapy continues to be regarded as the most viable option for treating cancer when surgery is not valid. In this context, our group has been working on the incorporation of the promising anticancer complex [Ru($\eta^5$-$C_5H_5$)(PPh$_3$)(2,2'-bipyridine)][CF$_3$SO$_3$] (TM34) into metallodrugs delivery systems [2]. Although bipyridine ligands are extensively exploited in designing inorganic or organometallic complexes for medicinal applications, in particular for cancer, the use of monosubstituted 2,2'-bipyridines remains underexploited, mostly due to sparce commercially available options and difficult synthesis.

Herein, we disclose the synthesis and structural characterization (NMR, UV-Vis, FT-IR) of a novel 4-monosubstituted 2,2'-bipyridine, as well as coordination of this ligand to a ruthenium cyclopentadienyl complex, that will be used as the cytotoxic agent of a smart metallodrug delivery system [2].



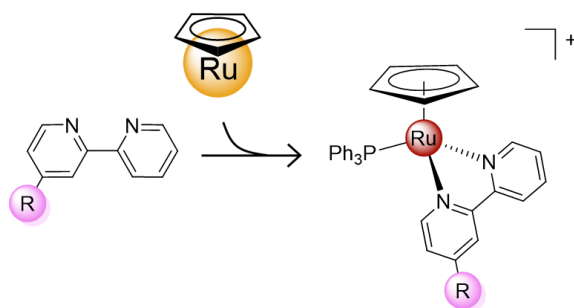**Fig. 1.** Strategy for the preparation of ruthenium complexes with a monosubstituted 2,2'-bipyridine ligand.

**References:** [1] Wild, E. et al. (2020) Weiderpass, World Cancer Report, 199; [2] Machado, J.F. et al. (2020) Dalton Trans., 49, 5974-5987.

# P11. Epoxidation of Styrene Derivatives with Fe₃O₄ Magnetic Nanoparticles Functionalized with Mo

Henriques, Ana C. (1); Nunes, Carla D. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
E-mail: fc53043@alunos.fc.ul.pt

Epoxides are extremely valuable intermediates in reactions in the fine chemicals and pharmaceutical industry. The most efficient way to synthesize these compounds is through the epoxidation of the corresponding olefin in the presence of an oxidant. In the present work, styrene, *trans*-β-methylstyrene and 4-chlorostyrene were the chosen olefins to study their epoxidation reaction using functionalized iron oxide magnetic nanoparticles (MNPs) as catalyst. MNPs are heterogeneous catalysts which provide the advantage of being easily removed from the reaction through filtration or centrifugation. Due to their nanosize, they have a larger surface area and, consequently, a higher activity.

The MNPs were prepared by a co-precipitation method using Fe(II) and Fe(III), followed by a silica coating, offering stabilization to the core and the possibility to graft a pyridine derivative ligand. To functionalize the surface, a $[MoI_2(CO)_3]$ complex was then coordinated. The success of these 4 steps was confirmed by SEM and TEM analysis, FTIR spectroscopy and powder XRD.

The catalytic tests were performed at 328 K and 353 K using TBHP and $H_2O_2$ as oxidants and $CH_2Cl_2$, $CH_3CN$, EtOH and toluene as solvents. The obtained results were very promising, showing higher conversion values when TBHP was the oxidant and with a temperature of 353 K. The MNPs also proved to be a more efficient catalyst for the epoxidation of *trans*-β-methylstyrene.



**Fig. 1.** Synthetic pathway adopted to prepare the MNPs.

# P12. Study of the correlation of environmental parameters in very large oceanic systems

Borges, Carlos (1); Silva, Ricardo (2); Carinhas, Dora (1,3); Palma, Carla (1)

(1) Instituto Hidrográfico, R. Trinas 49, 1249-093 Lisboa, Portugal
(2) CQE/Fac. Ciências da Univ. de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal
(3) IIFA/Univ. de Évora, Largo Marquês de Marialva, 7002-554 Évora, Portugal
E-mail: carlos.borges@hidrografico.pt

Oceanic water masses present some special characteristics, such as conservative oceanographic parameters like temperature and salinity that distinguish them from other water masses. Previous studies evidence the existence of relationships between nutrients and some of these conservative parameters, e.g. [1]. However, the determination of this correlation is affected by system heterogeneity and measurement (including sampling) uncertainty. This masking will, expectably, increase with the system's dimension and, hence, heterogeneity.

This work presents a tool developed to estimate the correlation between the values of a pair of parameters of very large marine systems estimated from a large environmental area considering the impact of system heterogeneity, sampling and analytical uncertainties in the assessment. The tool was applied to data from an area of the Portuguese coast with approximately 25 x 45 nautical miles containing 57 sampling stations.

The uncertainty of "representative" sampling was estimated from the Monte Carlo simulation of georeferenced information affected by analytical uncertainty [1] and produced, for each studied variable, more than 60 000 simulated results. The quantification of the correlation between pairs of variables was performed by Kendall's rank correlation coefficient, $\tau$ [2]. Each simulated pair of parametric values was obtained for the same GPS coordinates, in order to avoid losing or reducing observed correlation from system heterogeneity. It was assumed that data correlation is strong or very strong if the (absolute) calculated $\tau$ is between 0.7 and 1, moderate if $\tau$ is between 0.5 and 0.7 and weak to negligible if $\tau$ is 0.5 or smaller [3].

The correlations between several parameters were assessed. These were considered to be relevant regardless of system heterogeneity and analytical uncertainty for, e.g., the case of the pair *Conductivity-temperature* ($\tau$ = 0,690), but negligible in some other cases, e.g., *Conductivity-silica* ($\tau$ = -0,027).

**References:** [1] Borges, C. et al (2023) Evaluation of temporal trends and correlations of physical-chemical parameters in vast oceanic areas robust to information uncertainty. Chemosphere, 314, 137597; [2]; Kendall, M (1938) A New Measure of Rank Correlation. Biometrika, 30, 81-93; [3] Mukaka, M (2012) Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 24, 69-71.

# P13. Structural changes in iron(III) complexes and their impact on the spin crossover phenomenon

Gomes, Tiago (1); Ferreira, Liliana P. (2,3); Gomes, Clara S. B.(4); Xavier, Nuno (1); Martinho, Paulo N. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
(2) Biosystems and Integrative Sciences Institute (BioISI), Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa
(3) Department of Physics, University of Coimbra, 3004-516 Coimbra, Portugal
(4) LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal
E-mail: tpereiragomes@hotmail.com

In transition metal complexes, with iron species being a great example, the spin crossover effect possesses a strong impact on how the transition between spin states occurs, as well as on their respective electronic and structural properties [1]. Complementary studies suggested that potential modifications of the ligands or the anionic counterion in these complexes – one case being the presence of halogen atoms in the structure of N-ethyl-N-(2-aminoethyl)salicylaldiminate (SalEen) – affect the pattern of the magnetic profile obtained for these Fe(III) compounds [2]. Additionally, different polymorphs of the same compound can be obtained just by changing the solvent used for the crystallisation process conducted in the synthesis of these complexes or by controlling the evaporation rate of the crystallization solvent. [3,4]. With this in mind, we focus on the optimisation of previously studied Fe(III) complexes and the synthesis of newer ones, to extend the library of compounds under study – while also trying to obtain differing complexes, by varying the solvent of the crystallisation process.

**References:** [1] Nihei, M. et. al. (2007) Spin Crossover Fe(III) Complexes, Coor. Chem. Rev., 251, 2606-2621; [2] R. Marques et. al. (2022) The Halogen Effect on the Magnetic Behaviour of Dimethylformamide Solvates in [Fe(halide-SalEen)$_2$]BPh$_4$, Magnetochemistry, 8, 162; [3] Boonprab, T. et. al. (2018) Solvatomorphism and anion effects predominantly low spin iron(III) Schiff base complexes, Dalton Trans, 47, 12449; [4] Vicente, A. I. et al. (2018) Selecting the spin crossover profile with controlled crystallization of mononuclear Fe(III) polymorphs, Dalton Trans, 47, 7013-7019.

# P14. Highly Efficient Mechanochemical Synthesis of Hybrid and Metal-Free Perovskites

Bettencourt, Katherine (1); O. Feliciano, Inês (2); M. Piedade, M. Fátima (2)

(1) Chemistry and Biochemistry Department, Faculty of Sciences, Universidade de Lisboa.
(2) Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa.
E-mail: fc56587@alunos.fc.ul.pt

The irreversible changes global warming has caused to the environment led to climate shifts that have alarmed the entire international community. In an effort to mitigate these effects, the search for greener syntheses and energies gained prominence over the past two decades [1]. Regarding this matter, new renewable energy devices were developed, among which one device stands out due to its reliance on an extraordinary compound — perovskites.

Perovskites are remarkably versatile compounds derived from the parent formula $ABX_3$, as illustrated in Figure 1. They exhibit numerous interesting properties that have revolutionized various fields, including catalysis, LEDs, Solid Oxide Fuel Cells, and most recently, solar cells [2]. However, as with many remarkable discoveries, challenges lie in their synthesis. Traditional methods involve high temperatures, toxic solvents, and lengthy crystallization processes, resulting in low yields and purities in most cases [3]. To overcome these challenges, mechanochemistry has been used as an emerging tool for their synthesis, significantly reducing reaction times from days to minutes and improving low purities into meaningfully high purities, resulting in a fast, eco-friendly, and efficient method [3]. In this study, we have attempted to synthesize metal-free and hybrid perovskites using 1,4-diazabicyclo[2.2.2]octane (DABCO), ammonium salts and halogen inorganic salts such as $SnCl_2 \cdot 2H_2O$ and $SnI_4$.



**Fig. 1.** Perovskite structure.

**References:** [1] National Renewable Energy Laboratory, (2023) Best-Cell Efficiency Chart, NREL. [2] Arul, N.S, & Nithya, V.D. (Eds.) (2020) Revolution of Perovskite: Synthesis, Properties and Applications. Springer, 1-42 [3] Dulian, P. (2016) Solid-State Mechanochemical Syntheses of Perovskites. IntechOpen.

# P15. Oxidative Desulfurization of sulfur compounds present in oil tanks from refineries

Moreira, Pedro (1); Nunes, Carla (1)

(1) Centro de Química Estrutural – Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
E-mail: fc53071@alunos.fc.ul.pt

The increased awareness of the adverse effects of burning sulfur containing fuels on human health and environment has awakened the production of green fuels. It is imperative to remove these sulfur compounds in order to produce green fuel oils and meet the new requirements of sulfur standard contents (10-15 ppm). [1-2] With that, oxidative desulfurization (ODS) has been considered a promising and highly efficient method owing to its mild operation conditions and high efficiency. ODS includes two steps: (1) an appropriate agent oxidizes the organic sulfur compounds to sulfoxides and/or sulfones; (2) the oxidation products are removed by suitable methods. In this work, a new material was developed and characterized to be used as a catalyst in ODS desulfurization of sulfides. The catalyst was prepared by supporting the precursor complex $MoI_2(CO)_3(MeCN)_2$ on iron oxide nanoparticles shelled with silica and modified with an organic moiety. The $NPM_{30}$-Si-inic-Mo nanomaterial was obtained and characterized by infrared spectroscopy (FTIR) and X-ray powder diffraction (DRX) and by Scanning Electron Microscopy (SEM) and Transmission (TEM). The $NPM_{30}$-Si-inic-Mo was then tested as catalyst in the oxidation of four sulfides. All reactions were carried out at 80 °C, varying the oxidant as well as the substrate:oxidant ratio. In general, it was found that the oxidation to sulfoxide and sulfone occurred, and the most promising oxidant to obtain the sulfone was TBHP. The catalytic tests revealed promising results, and the possibility of recovering the catalyst in some reactions, through a magnet, was tested, in which it was verified that, after its removal, the resulting solution was clear.
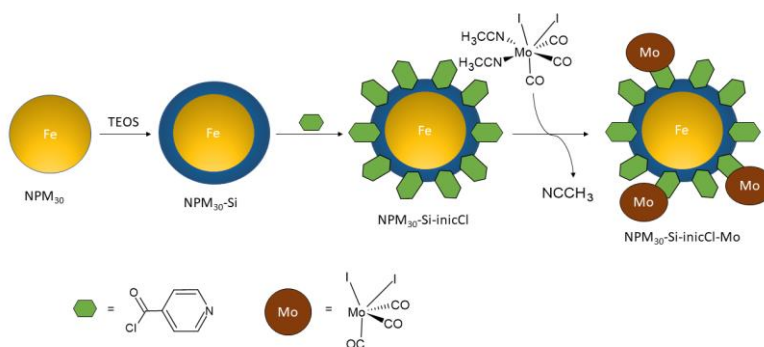


**Fig. 1.** Catalyst synthesis steps

**References:** [1] Hossain, M.N. et al. (2019) Catalysts, 9, 229. [2] Rajendran, A. et al. (2020) J. Mater. Chem. A, 8, 2246–2285.

# P16. $CO_2$ photoreduction with Co(II) and Co(III) coordination compounds

Paes, Eduardo A (1); T. Marques, Rafaela (1); Bento, Marcos (1); S.B. Gomes, Clara (2); N. Martinho, Paulo (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
(2) Centro de Química Estrutural - Institute of Molecular Sciences, NOVA School of Science and Technology, NOVA University Lisbon
E-mail: fc56609@alunos.fc.ul.pt

With the continuous increase of global warming and alarming climate changes due to the rise of greenhouse gases emissions, such as $CO_2$, mainly caused by the colossal industrial pollution, different methods of carbon capture and utilization have been proposed to solve this urgent matter.

Catalysis has been an attractive approach to the conversion of $CO_2$ into molecules with added value, such as, CO, $CH_4$, HCOOH[1] and $CH_3OH$[2]. The homogeneous photoreduction of $CO_2$ is composed by a catalyst (that in the active form converts the $CO_2$), a sacrificial electron donor (that donates electrons, and it is stoichiometrically consumed) and a photosensitizer (that absorbs light and mediates the electron transfer between the catalyst and the sacrificial donor). Several compounds using different metallic centers (usually transition metals) and different ligands have been studied in the photoreduction of $CO_2$. Among the transition metals, Mn, Re, Fe, Co and Ni are the most commonly used in as catalysts for the photoreduction of $CO_2$ [2].

We present the synthesis and characterization of different Co(II) and Co(III) complexes based on different imine and amine ligands. These compounds were analyzed by Fourier Transform Infrared spectroscopy (FTIR) and Ultraviolet-Visible spectroscopy (UV-vis), cyclic voltammetry (CV) and nuclear magnetic resonance (NMR). We also studied their ability for $CO_2$ photoreduction.

**References:** [1] Call, A. et al. (2019) ACS Catalysis, 9, 4867-4874; [2] Realista, S. et al. (2019) Co(II) cryptates convert $CO_2$ into CO and $CH_4$ under visible light, Chemistry - A European Journal.

# P17. Tracking trace levels of β-blockers in aqueous matrices by bar adsorptive microextraction (BAµE)

Mendes, Mariana (1); Neng, Nuno (1); Nogueira, José (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
E-mail: fc51347@alunos.fc.ul.pt

β-blockers, a class of drugs commonly prescribed for the treatment of cardiovascular diseases, but also used in other therapies, namely to control anxiety, were banned by the World Anti-Doping Agency (WADA) in several sports given their great effect in reducing tremors and blood pressure [1]. Moreover, these molecules have been detected in wastewater and environmental matrices above the predicted no-effect concentration, due to their high worldwide consumption and the human body inability to completely metabolize them [2]. Therefore, developing alternative analytical methodologies to determine trace levels of β-blockers in biological and environmental matrices becomes an essential issue for toxicology, therapeutic monitoring, doping control, and environmental contamination analysis.

The present work aims to track trace levels of β-blockers (atenolol, bisoprolol, carvedilol, nebivolol, pindolol and propranolol) in aqueous matrices, using an alternative analytical approach, bar adsorptive microextraction followed by high performance liquid chromatography with diode array detection (BAµE/HPLC-DAD) [3]. This methodology was developed and optimized by evaluating several important parameters during the microextraction (type of sorbent phase, equilibrium time, matrix pH and ionic strength) and back-extraction stages, for achieving high recoveries (71.8 - 90.4 %; RSD < 13.5 %). Additionally, it is also our intention to apply the optimized and validated methodology to real matrices, *i.e.*, urine and wastewater samples.

**References:** [1] Yıldırım, S. et al. (2022) Novel Trends in Analytical Methods for β-Blockers: An Overview of Applications in the Last Decade. Crit Rev Anal Chem, 52, 131-169; [2] Iancu, V. et al. (2019) A new analytical method for the determination of beta-blockers and one metabolite in the influents and effluents of three urban wastewater treatment plants. Journal Anal Methods, 11, 4668-4680. [3] Neng, N. et al. (2010) Adsorptive microextraction techniques - Novel analytical tools for trace levels of polar solutes in aqueous media. J Chromatogr A, 1217, 7303-7310.

# P18. Treatment of effluents containing hexavalent chromium by electroless precipitation on polyaniline films

Morais, Carolina (1); Correia, Jorge (1); Oliveira, Cristina (1)

(1) Departamento de Química e Bioquímica; Centro de Química Estrutural; Institute of Molecular Sciences, FCUL, Campo Grande, Lisboa
E-mail: fc56603@alunos.fc.ul.pt

Electroless precipitation is a metal ion reduction process carried out by some conductive polymers containing nitrogen atoms. It consists in a spontaneous reduction process, without using an electrical source of energy, exclusively of ions with a high potential reduction potential. [1]

The objective of this work is to study the performance of polyaniline films in the electroless precipitation of hexavalent chromium. These films were electrosynthesized on graphite electrodes in potentiodynamic mode.

Three polyaniline films were synthesized, all of them with the same number of cycles and the same sweep rate (50 cycles at 20 mV/s). The only variable was the type of electrode where the film was formed. Graphite electrodes with different porosities were used in order to select which one produced a more reliable film with higher electroactivity, that reduced a greater amount of hexavalent chromium.

The thickness of the film and their dielectric properties were accessed by ex-situ ellipsometry using a conventional three phase model in a multi incident angle approach.

To evaluate the performance of polyaniline films in the reduction of $Cr^{VI}$ to $Cr^{III}$, samples collected during electroless precipitation were complexed with 1,5-Diphenylcarbazide (DFC) and analyzed by UV-Vis spectroscopy. [2]

Finally, in order to examine the robustness of the polymer, reuse trials were made with the film that had revealed the best reduction efficiency. With these tests it was possible to determine the number of times that polyaniline was able to reduce hexavalent chromium without losing its properties and compromising its efficiency. [2]
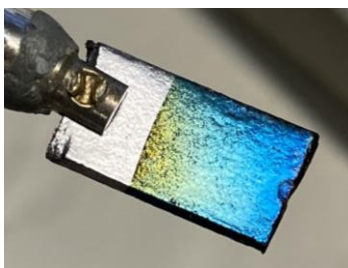


**Fig. 1.** Polianiline film in its most oxidized state on a graphite electrode.

**References:** [1] Mourato, A. et al. (2004) Electrochim. Acta, 49, 2249–2257; [2] Canhoto, M. F. Avaliação do desempenho de filmes de polímeros eletronicamente condutores na redução de iões metálicos potencialmente poluentes. Relatório de projeto da Licenciatura em Química. FCUL **2022**.

# P19. Adhesive and conductive co-polymers for biosensing applications

Carneiro, Diana M. (1); Zeferino, Jorge F. (1); Almeida, Luís C. (1); Correia, Jorge P. (1); Viana, Ana S. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
E-mail: dianamcarneiro@alunos.fc.ul.pt

The synergistic effect of the combination of cathecol and amine groups provides interesting adhesive properties to polydopamine (PDA), a biomimetic polymer inspired by the mussel's foot proteins. This versatile polymer allows the coating and functionalization of practically all types of surfaces, having great application in several areas, such as biomedicine, nanotechnology, and (bio)sensing [1,2]. However, PDA is a chemically heterogeneous and poorly conductive polymer, which limits its electrochemical utility, including its use in amperometric biosensors [3,4]. To overcome this limitation, this work aims to optimize and explore the electro co polymerization of two monomers, dopamine (DA) and pyrrole (Py); the latter known to origin a highly electronically conducting polymer – polypyrrole (PPy). The combination of PDA's adhesivity, for further functionalization (*e.g.*, biomolecules) with the high conductivity of PPy, useful in electron transfer events, in a single polymer is expected to have an exceptional impact on the use of polycatecholamines in biosensing interfaces and electrocatalytic applications.

Potentiodynamic and potentiostatically grown co-polymers (PDA/PPy) were fully characterized, regarding their electrochemical behavior, optical properties, thickness, morphology, and wettability, by using a combination of electrochemical and surface characterization techniques (ellipsometry, electrochemical quartz crystal microbalance, atomic force microscopy, water contact angle measurements and FTIR). Overall, the co-polymers, *ca.* 30 nm thick, revealed superior electrochemical response towards negatively and positively charged ionic probes and towards target phenolic compounds when compared to pure polycatecholamine films.

Laccase was successfully immobilized on the co-polymer functional groups and the chronoamperometric assays proved that the modified electrode was sensitive for the detection of gallic acid (a model phenolic compound). The catalytic properties of these adhesive and conducting matrices were greatly improved.

**References:** [1] Ryu, J. H. et al. (2018) Polydopamine Surface Chemistry: A Decade of Discovery. ACS Appl. Mater. Interfaces, 10, 7523-7540; [2] Almeida, L. C. et al. (2019) Electrochemical deposition of bio-inspired laccase-polydopamine films for phenolic sensors. Electrochim. Acta, 319, 462-471; [3] Almeida, L. C. et al. (2021) Comprehensive study of the electrochemical growth and physicochemical properties of polycatecholamines and polycatechol. Electrochim. Acta, 386, 138515; [4] Almeida, L. C. et al. (2022) Combined Electrochemical, Ellipsometric and Microgravimetric Study of Ion Permeable Polydopamine Films. J. Electrochem. Soc., 169, 046503.

# P20. The Photo- and Thermo-Salient Effect in Fe(III) spin crossover complexes

Fernandes, André (1); Bento, Marcos A. (1); Gomes, Clara S. (3) B.; Botelho, Hugo M. (2); Martinho, Paulo N. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa.
(2) BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa.
E-mail: fc55342@alunos.fc.ul.pt

Numerous technical applications can make use of multifunctional materials. Different sorts of molecules, such as metal complexes, may serve as the foundation for these materials. When exposed to an external stimulus, such as a change in temperature, pressure, or light irradiation, compounds that exhibit the spin crossover (SCO) phenomenon exhibit altered magnetic behavior. Fe(III) complexes with Schiff base ligands are an example of a chemical that exhibits the SCO phenomenon. These complexes can form crystals with spin lability and possess photo- and thermo-responsive characteristics. These thermo- and photo-responsive qualities result from their distinct molecular structure, which enables physical changes in response to changes in temperature and light exposure. These modifications impact their volume, shape, and mechanical characteristics, leading to flexible crystals that directly convert light and heat energy into mechanical work. Our strategy involves using Fe(III) complexes to produce molecules that are mechanically responsive and have magnetic switching capabilities, combining these mechanical effects with the SCO phenomenon in the same material. Here, we present the results of optical microscopy-based characterisation of Fe(III) SCO complexes with thermal and photosalient characteristics.
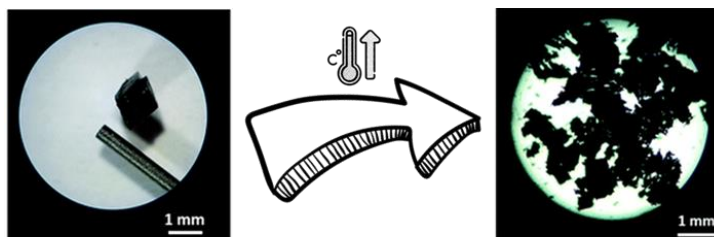


**Fig. 1.** Thermosalient effect in an Fe(III) SCO compound.

**References:** [1] Yang, Y. et al. (2022) Photomechanical crystalline materials: new developments, property tuning and applications. CrystEngComm, 24, 3136; [2] Petra J. et al. (2004) Iron(III) Spin Crossover Compounds. Top Curr Chem 2004, 233, 259–324; [3] Zhuo, Z. et al. (2022) Research progress of mechanically flexible molecular crystals: From bending mechanisms to applications. Chem. Eng., 450, 138333.

# P21. Metal-organic framework films as uric acid sensors

Alonso, Rui D.(1); Reis, Ana R.(1); Ferraria, Ana M. (2); Botelho do Rego, Ana M. (2); Martinho, Paulo N. (1); Realista, Sara (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
(2) BSIRG, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory i4HB - Institute for Health and Bioeconomy, and Chemical Engineering Department at Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal
E-mail: fc54695@alunos.fc.ul.pt

Uric acid is a molecule with a high role in medical applications since its abnormal concentration translates into a diagnosis of clinical diseases. Therefore, the development of purine sensors became extremely important and relies on the fabrication of thin films based on nanomaterials, like metal-organic frameworks. [1,3]

Metal-organic frameworks (MOFs) consist of organic linkers and a metal ion and have been intensively studied as catalysts, sensors and in molecular separation and storage due to their properties such as high porosity, crystallinity and the presence of several active sites. [2]

In our research, we focused on Fe-MOF-74 and on the development of films using a direct electrochemical deposition technique - cathodic deposition - that uses the MOF precursors directly.To produce the films, we used two different organic linkers, one of them commercially available and the other one was synthetised and characterised by nuclear magnetic resonance spectroscopy.

The films obtained were characterised by diffuse reflectance infrared fourier transform spectroscopy (DRIFT), X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). The new films were used for the electrochemical sensing of uric acid.
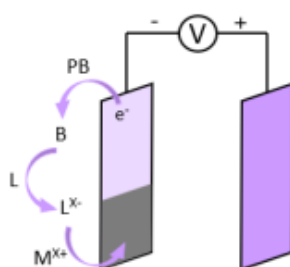


**Fig. 1.** Electrochemical MOF film fabrication method, cathodic deposition

**References:** [1] Liu, L. et al. (2019) Talanta, 199, 478; [2] Bláha, M. et al. (2020) J. Phys. Chem. C, 124, 24245; [3] Sharma, V. K. et al. (2015) Sensors, 15, 1564.

# P22. Objective determination of trends of microplastic contamination in a vast Atlantic Ocean area

Morgado, Vanessa (1,2); Palma, Carla (2); Bettencourt da Silva, Ricardo (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
(2) Instituto Hidrográfico
E-mail: vmmorgado@fc.ul.pt

The global awareness of environmental pollution caused by microplastics is widely acknowledged. Microplastics have been detected in various environmental compartments worldwide, including remote areas. Different methodologies and techniques have been employed to analyse microplastics in these matrices, such as sediment samples from the ocean bottom. Typically, in order to identify microplastics in sediment, the sample is sieved through a 5 mm mesh, digested to eliminate organic matter, and subjected to density separation to isolate microplastics from the denser sediment fraction [1]. The physical analysis of microplastics involves visual examination under a stereomicroscope to determine their size, colour, and shape. Chemical analysis is carried out using an infrared spectrometer coupled with a microscope (micro-FTIR), enabling the identification of the polymer's chemical composition.

Implementing policies and legislation to control and manage plastic pollution, including microplastics, is crucial for safeguarding the environment, particularly coastal areas. The development of regulations should be informed by the recognised significance and trends of this form of pollution. This study focuses on evaluating contamination trends in a 700 km$^2$ oceanic area characterised by heterogeneous contamination, representativeness of sampling, and uncertainties associated with sample analysis [2]. The developed methodology involves objectively identifying substantial variations in microplastic contamination through Monte Carlo simulation, considering all sources of uncertainty. This investigation unequivocally demonstrates that the contamination level in the studied area did not significantly change between two consecutive years (2018 and 2019), with PET microplastics being the predominant polymer type. The comparison of contamination levels was conducted with a 99% confidence level. The collected data pertaining to the environmental area play a crucial role in establishing an objective and binding assessment of microplastic contamination significance [3].

**References:** [1] Morgado, V. et al. (2022) Microplastics contamination in sediments from Portuguese inland waters: Physical-chemical characterisation and distribution. Sci. Total Environ., 832, 155053; [2] Morgado, V. et al. (2022) Bottom-up evaluation of the uncertainty of the quantification of microplastics contamination in sediment samples. Environ. Sci. Technol., 56, 11080-11090; [3] Morgado, V. et al. (2023) Determination of microplastic contamination levels and trends in vast oceanic sediment areas with uncertainty. Sci. Total Environ., 884, 163612.

# P23. $CO_2$ photoreduction using cryptates with Earth abundant metals

Marques, Rafaela T.(1); Realista, Sara (1); Santos, Rui G (2); Martinho, Paulo N. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
(2) CERENA - Centre for Natural Resources and the Environment, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
E-mail: rfmarques@fc.ul.pt

$CO_2$ plays a crucial role in the carbon cycle, which keeps the Earth's temperature stable. The expansion of the human population and the energy demand, increased Earth's $CO_2$ concentration unbalancing the carbon cycle, affecting our planet's energy balance. This led to the urgency of finding efficient pathways of carbon utilisation and recycling to form valuable products. Molecular activation is crucial in chemical and biological systems, where $CO_2$ is one important player. Thus, researchers and industries had a deep interest in creating catalysts that, by electro- and photoreduction, can convert $CO_2$ either into liquid fuel precursors (carbon monoxide and hydrogen) [1] or directly to liquid fuels (methanol and/or methane). [2] The photoconversion of $CO_2$ can be made in homogeneous and heterogeneous media. The former has the advantage of modulating the catalytic active sites to improve selectivity. It requires three components: the catalyst (CAT, which in the active form, converts $CO_2$), the sacrificial donor (SD, donates electrons and is consumed) and the photosensitiser (PS, absorbs light and mediates the electronic transfer between the CAT and the SD).

Our research group reported Co(II)-cryptates, catalysts, with different substituents in the aromatic rings (-Br, -NO₂, -CCH) and observed that the capture and conversion of $CO_2$ were affected by them. [3] We present the synthesis and characterisation of Co(II)/Co(II), Co(II)/Zn(II) and Fe(II)/Zn(II) cryptates previously synthesized and new ones with -Br substituent in the aromatic rings. The photoreduction of $CO_2$ and the photocatalytic system and setup was also investigated.
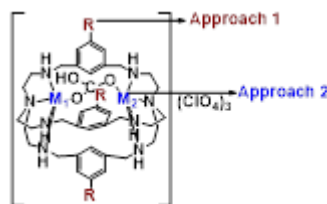


**Fig. 1.** Dinuclear Co(II)/Co(II), Co(II)/Zn(II) or Fe(II)/Zn(II) cryptate with $ClO_4^-$ as anion.

**References:** [1] Sheng, W. et al. (2017) Energy Environ. Sci., 10, 1180–1185; [2] Kuhl, K. P. et al. (2014) J. Am. Chem. Soc., 136, 14107–14113; [3] Realista, S. et al. (2019) Chem. Eur. J., 25, 11670–11679.

# P24. Synthesis of propargylamines using green methodologies *via* A$^3$ Coupling catalyzed by Copper(I) complexes

Costa, Diogo (1); Gomes, Clara S.B. (1); Rosa, Vitor (1)

(1) LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology (FCT-NOVA), NOVA University Lisbon 2829-516 Caparica, Portugal.
E-mail: dmc.costa@campus.fct.unl.pt

Propargylamines are a class of versatile compounds that contain a triple bond and an amino group in their structure, giving them a high reactivity that makes them valuable building blocks for the synthesis of various molecules such as heterocycles and alkaloids, which play an important role in medicinal chemistry and in the pharmaceutical industry, due to their biological activity.

There are several approaches to the synthesis of propargylamines, such as amination of propargyl moieties and alkynylation of imines, and in recent years there has been progress in a method called A$^3$ coupling, which consists of a one-pot reaction of aldehydes/alcohols, amines and alkynes catalyzed by transition metals. [1]

However, most of the processes require the use of high temperature, harsh reaction conditions and high catalyst loading. In this work, we developed a green and sustainable way to synthetize propargylamines via one-pot coupling of aromatic alcohols/aldehydes, aliphatic/aromatic amines and aliphatic/aromatic alkynes using copper(I) homoleptic and heteroleptic complexes, using bis(imino)acenapthenes (BIANs) and BIAN-NHCs (N-Heterocyclic Carbenes) as ligands, providing control and stability to the metal core.



**Cu(I) catalysts** (R = H, Me, *i*-Pr, OMe; X = Cl, Br, I, OTf, BF$_4$, PF$_6$)
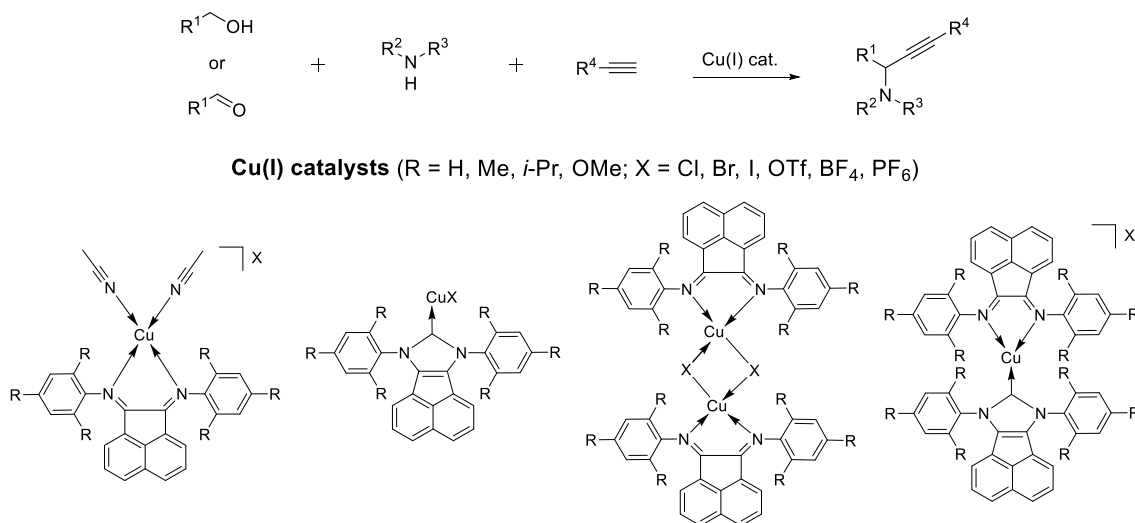
**Fig. 1.** Scheme of the A$^3$ coupling reaction and the family of catalysts used in this work.

**References:** [1] Jesin, I et al. (2019) Recent Advances in the A$^3$ Coupling Reactions and their Applications. EurJOC, 16, 2704-2720;

# P25. Functionalization of Natural Bisquinolizidine Alkaloids

Muiz, Abdullahi A. (1); Durão, Raquel (1,2); Coelho, Jaime A. S. (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
(2) Instituto de Investigação do Medicamento, Faculdade de Farmácia, Universidade de Lisboa.
E-mail: aamuiz@fc.ul.pt

Bisquinolizidine alkaloids (BQA) are found in several plants of the subfamily Faboideae. Structurally, they contain a chiral bispidine core decorated with fused N-annulated piperidinone or piperidine moieties [1]. An important member of the group is sparteine, which is commonly used as a chiral ligand for various metals in asymmetric synthesis [2]. However, the limited reactive functional groups on sparteine and other BQA pose a functionalization challenge. Thus, limiting their use in metal-free organocatalysis.

Taking advantage of the recent advances in electrochemical organic synthesis that enables gram scale reactions, a site-selective electrochemical C-H activation was explored, and several functional group transformations are currently being investigated (Fig. 1) [3].
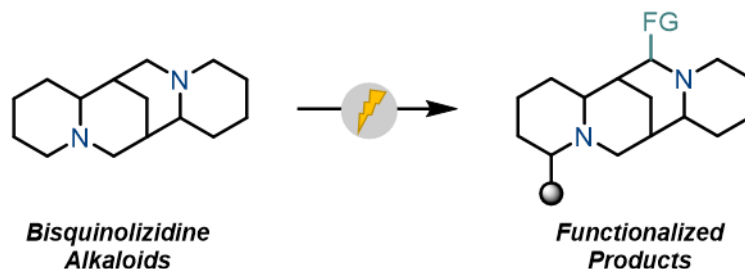


**Fig. 1.** Electrochemical functionalization of bisquinolizidine alkaloids.

**References:** [1] Michael, J.P. (2016) Simple Indolizidine and Quinolizidine Alkaloids, Hans-Joachim Knölker (Ed), The Alkaloids: Chemistry and Biology, Academic Press, 75, 1-498; [2] Chuzel, O et al. (2005) Sparteine as a Chiral Ligand for Asymmetric Catalysis. In: Lemaire M., Mangeney P. (eds) Chiral Diazaligands for Asymmetric Synthesis. Topics in Organometallic Chemistry, 15. Springer, Berlin, Heidelberg; [3] Kärkäs, M.D. (2018) Electrochemical strategies for C−H functionalization and C−N bond formation. Chem. Soc. Rev, 47, 5786-5865.

# P26. Novel ruthenium-peptide conjugate for targeting metastatic breast cancer

Sá, Marco (1); Tarita, Miguel (2); Coelho, Jaime A. S. (1) Marques, Fernanda (2); Garcia, M. Helena (1); Correia, João D. G. (2); Morais, Tânia S. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
(2) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10 (km 139, 7), 2695-066 Bobadela LRS, Portugal.
E-mail: marcosa@alunos.ciencias.ulisboa.pt

Metastatic breast cancer (MBC) is a highly aggressive subtype of breast cancer that accounts for 15-20% of all breast cancer cases. Unfortunately, there is still no clinical cure for this subtype of cancer, and available treatments have limited effectiveness and often cause severe side effects due to their lack of specificity [1]. To overcome the limitations of existing therapies, our group is currently developing novel ruthenium smart metallodrug delivery systems capable of targeting both primary tumor and metastases of breast cancer [2]. These systems comprise a peptide that recognizes with high affinity the fibroblast growth factor receptor (FGFR), often overexpressed by MBC cells, linked to a cytotoxic ruthenium-cyclopentadienyl complex through a pH-sensitive function that responds to the slightly acidic tumor microenvironment. These systems allow accumulation, site- and time-specific release of the active species into the tumor (Fig. 1).

Herein, we report the synthesis, characterization, and biological evaluation of a new pH-responsive ruthenium-peptide conjugate (RuPC) to be used as a smart metallodrug delivery system for MBC therapy. A new RuPC was synthesized and fully characterized for the first time. The drug release profile was evaluated in solution at pH values that mimic the tumor microenvironment and the bloodstream. The *in vitro* cytotoxicity of the conjugate and the free complex was evaluated in four human breast cancer cells lines with different levels of FGFR expression.
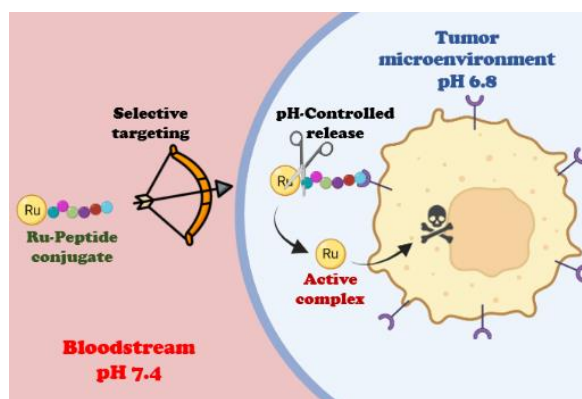


**Fig. 1.** Proposed mechanism of action of the ruthenium-peptide conjugate.

**References:** [1] Yin, L. et al. (2020) Triple-negative breast cancer molecular subtyping and treatment Breast Cancer Res., 22, 1-13; [2] Machado, J. et al. (2020) Novel "ruthenium cyclopentadienyl"–peptide conjugate complexes against human FGFR(+) breast cancer. Dalton Trans., 49, 5974-5987.

# P27. Enhancing Active Pharmaceutical Ingredients Physical Properties via Cocrystal Formation

Monteiro, Tomás (1); Feliciano, Inês O. (1); Bernardes, Carlos E. S. (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa
E-mail: fc56606@alunos.fc.ul.pt

Active Pharmaceutical Ingredients (APIs) solubility is a major concern during the development of drug formulations since it directly affects a substance's capacity to enter the human bloodstream after consumption. In the last decades, several strategies have emerged to address this issue, many of them relying on changes in the molecular organization of the drug in the solid state, leading to the establishment of a new field of research known as Crystal Engineering [1]. In this way, it is expected to control the intermolecular forces of the API in the crystal, which ultimately impacts its physical properties (e.g., solubility). Among the developed methodologies, cocrystallization (i.e., the preparation of crystalline solid materials in which the API is combined with other organic molecules) has become one of the most promising methodologies, as a judicious selection of a co-former allows the fine-tuning of the API physical properties.

Nicotinamide (NIC) is a form of vitamin B3 found in food and used as medicine due to its anti-inflammatory properties. Additionally, is often selected to produce cocrystals of other APIs due to its ability to form different types of hydrogen bonds. It is, therefore, an ideal candidate for systematic studies on the formation of this type of materials. Thus, the work here reported is part of ongoing research aiming the investigation of the co-crystallization of NIC with dicarboxylic acids (ADs), by evaluating how the solubility and the stability of the produced materials vary as the co-former is systematically changed in different stoichiometric quantities. The obtained materials were characterized by powder X-ray diffraction (XRPD) and differential scanning calorimetry (DSC). In this studies was synthesised cocrystals of NIC with succinic acid (SUC), oxalic acid (OXA), malonic acid (MAL), glutaric acid (GLU) and pimelic acid (PIM). Furthermore it was shown that it is possible to decrease the solubility of NIC by producing a co-crystal with SUC ($NIC_2$:SUC). Using the solubility values obtained the Gibbs free energy was determined for the reaction $NIC_2$:SUC (cr) $\rightarrow$ 2NIC (cr) + SUC (cr), $\Delta_r G_m^o$ =13.9 kJ·mol$^{-1}$, indicating that the cocrystal is stable relative to the precursors.

**References:** [1] Bavishi, D.D. (2016) Spring and parachute: How cocrystals enhance solubility. Prog Cryst Growth Charact Mater. 62, 1-8.

# P28. Study of the influence of the composition of a mixture of fruits and vegetables on the content of total phenolics, flavonoids and vitamin C

Salgado, Neuza (1,2); Silva, Mafalda Alexandra (2,3); Costa, Helena S. (2,3); Figueira, Maria Eduardo (4) Albuquerque, Tânia Gonçalves (2,3)

(1) Faculty of Pharmacy, University of Lisbon
(2) Research and Development Unit, Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge
(3) REQUIMTE-LAQV/Faculty of Pharmacy, University of Oporto
(4) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon
E-mail: neuzaigsalgado@gmail.com

Low consumption of fruits and vegetables is among the top ten risk factors that contribute to global mortality. According to the World Health Organization and Food and Agriculture Organization of the United Nations, a minimum consumption of 400 g of fruit and vegetables per day (excluding starchy tubers) is recommended, in order to prevent obesity, diabetes, cancer, and cardiovascular diseases, among others. Detox juices, a mixture of fruits and vegetables, can probably be useful to increase the intake of fruits and vegetables. However, the composition of the mixture, as well as the amount of each ingredient, are crucial to maximize the health potential of the juice, namely in terms of antioxidants availability. In this study, strawberry (SA), raspberry (R), blueberry (B), lettuce (L) and spinach (SP) were selected as ingredients to prepare five mixtures, with the aim of studying the influence of the composition of the mixtures on their total phenolics, flavonoids and vitamin C contents. In May 2023, the ingredients were acquired in different commercial surfaces of Lisbon region and when applicable the non-edible portions were discarded. A portion of each ingredient was used to prepare five mixtures (M1-M5). The amount of each ingredient varied between 10 and 50 g. Total phenolics and flavonoids were determined by spectrophotometric methodology, while vitamin C was determined by high-performance liquid chromatographic coupled to photodiode array detection. M4 (50%SP; 18.8%L; 11.2%SA; 10%B; 10%R) has the highest flavonoids content (303 ± 18.4 mg of epicatechin equivalents/100 g), while M2 (50%R; 18.4%L; 11.6%SP; 10%SA; 10%B) has the highest amount of total phenolics (322 ± 26.2 mg of gallic acid equivalents/100 g). Concerning total vitamin C, the content in the analysed mixtures varied between 1.96 ± 0.04 and 9.93 ± 0.08 mg/100 g). M2 was the one with the highest total phenolics and ascorbic acid contents but it has the lowest flavonoids content. In the next steps of this research work, other analytical parameters will be studied, to evaluate which is the best formulation of the mixture for consumers, from a public health point of view.

# P29. Light-induced isomerization of azophenyl moiety in SCO active Fe(II) complexes

Sagatova, Alexandra (1); Orvos, Jakub (2); Salitros Ivan (1)

(1) Department of Inorganic Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, Bratislava SK-81237, Slovakia
(2) Department of Organic Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, Bratislava SK-81237, Slovakia
E-mail: alexandra.sagatova@stuba.sk

The octahedral Fe(II) complexes of N-donor ligands show magnetic bistability, known as the spin crossover process (SCO)[1], which can be controlled by external stimuli such as temperature or light. The spin state transition between low-spin (LS) and high-spin (HS) states is based on a change in the ligand field strength. The main parameters characterizing the SCO are the magnetic profile and its transition temperature ($T_{1/2}$).[2] The light-induced SCO controlled upon isothermal conditions by ligand's moiety photoisomerization is known as the ligand-driven light-induced spin change (LD LISC) effect [3].

The primary purpose was to prepare the SCO active Fe(II) complexes with bidentate N-donor organic ligands based on the pyridylbenzimidazole with a photoactive azophenyl moiety. The *meta*- or *para*-position of the photoactive substituent on pyridyl caused a change in the magnetic profiles of the Fe(II) complexes and it has influenced also a yield in *trans-cis* photoconversion. While the $[Fe(L1)_3](CF_3SO_3)_2$ (L1 = *trans*-2-[4-(phenyldiazenyl)pyridin-2-yl]-1*H*-benzo[*d*]imidazole) has $T_{1/2}$ situated at 225 K, in the case of the $[Fe(L2)_3](CF_3SO_3)_2$ (L2 = *trans*-2-[5-(phenyldiazenyl)pyridin-2-yl]-1*H*-benzo[*d*]imidazole) was observed a shift of $T_{1/2}$ to the room temperatures region (283 K). The ^{1}H-NMR spectroscopy was used for the investigation of the yield of *trans-cis* conversion of both complexes in DMSO solution during one hour of 365 nm light irradiation. It shows that the $[Fe(L1)_3](CF_3SO_3)_2$ complex was successfully converted in 31 % yield, while the photoisomerization for the $[Fe(L2)_3](CF_3SO_3)_2$ complex reached 40 % new *cis* phase. The photoconversion of both complexes was also studied in solid state and based on the UV-VIS spectra of thin films prepared by the spin counting method. The photoisomerization induced by light of 365 nm wavelength showed that $\pi \rightarrow \pi^*$ bond was decreased and $n \rightarrow \pi^*$ was increased so much more for the $[Fe(L2)_3](CF_3SO_3)_2$ complex as the $[Fe(L1)_3](CF_3SO_3)_2$ complex.

**References:** [1] König, E. et al. (1966) Unusual magnetic behavior of some iron(II)−bis-(1,10-phenanthroline) complexes. Chem. Commun., 3, 61-62; [2] Gütlich, P. et al. (1994) Thermal and Optical Switching of Iron(II) Complexes. Angew. Chem. Int. Ed. Engl. 33, 2024–2054; [3] Roux, C. et al. (1994) Toward Ligand-Driven Light-Induced Spin Changing. Influence of the Configuration of 4 Styrylpyridine (stpy) on the Magnetic Properties of FeII(stpy)4(NCS)2 Complexes. Crystal Structures of the Spin-Crossover Species Fe(trans-stpy)4(NCS)2 and of the High-Spin Species Fe(cis-stpy)4(NCS)2. Inorg. Chem., 33, 2273–2279.

# P30. Analysis of total vitamin C, ascorbic acid and dehydroascorbic acid in red fruits and green leafy vegetables by HPLC-PDA

Salgado, Neuza (1,2); Silva, Mafalda Alexandra (2,3); Costa, Helena S. (2,3); Figueira, Maria Eduardo (4) Albuquerque, Tânia Gonçalves (2,3)

(1) Faculty of Pharmacy, University of Lisbon
(2) Research and Development Unit, Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge
(3) REQUIMTE-LAQV/Faculty of Pharmacy, University of Oporto
(4) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon
E-mail: neuzaigsalgado@gmail.com

Ascorbic acid (AA), also known as vitamin C, is an essential micronutrient due to its role in human development and wellbeing since it has antioxidant properties and is involved in the production of proteins like collagen. [1] It has been associated with several health benefits: reduced risk of mortality, cardiovascular disease, different types of cancer, and respiratory and neurological issues. [2] Despite being mainly found in citrus species, AA also exists in other fruits and vegetables. This study aimed to assess the variability of vitamin C content in red fruits (strawberry, raspberry and blueberry) and green leafy vegetables (spinach and lettuce) from different brands and supermarkets, measuring total vitamin C, by high-performance liquid chromatography with photodiode array detection (HPLC-PDA), an extremely sensitive and validated method.

According to our results, brand B of strawberry was the richest in total vitamin C (51.3 mg/100 g) with 45 mg/100 g of AA and 6.27 mg/100 g of DHA, followed by brand A of raspberry (44.7 mg/100 g), being the majority AA (38 mg). On the contrary, all brands of blueberry did not contain any form of vitamin C and brand B of lettuce had 5.01 mg/100 g of total vitamin C, reporting the lowest detectable value. Within the different brands, total vitamin C ranged between 37.4-51.3, 20.8-44.7, 5.01-5.13 and 10.1-12.4 mg/100 g for strawberry, raspberry, lettuce and spinach, respectively. Thus, red berries seem to have higher quantity of vitamin C than green leafy vegetables, except for blueberry. Differences between the same food of distinct brands can be justified due to cultivation practices, ripeness, environmental and soil conditions, as well as storage conditions since vitamin C is sensitive to heat, light, and oxygen. Furthermore, consulting Regulation Nº 1169/2011, which indicates 80 mg of vitamin C as daily reference dose for adults, consuming 1 portion (160 g) of brand B of strawberry will make you achieve 103% of the recommendation.

**References:** [1] Santos, K. et al. (2022) Essential features for antioxidant capacity of ascorbic acid (vitamin C). J Mol Model., 28, 1-8; [2] Xu, K. et al. (2022) Vitamin C intake and multiple health outcomes: an umbrella review of systematic reviews and meta-analyses. Int J Food Sci Nutr., 73, 588-599.

# P31. Development of new halogenated isoniazid derivatives with antitubercular properties via computational methods

Francisco Duarte (1,2); Filomena Martins (2); Miguel Machuqueiro (1)

(1) Biosystems and Integrative Sciences Institute (BioISI), Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa
(2) Centro de Química Estrutural - Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa.
E-mail: fpduarte@ciencias.ulisboa.pt

Tuberculosis (TB) is the second leading cause of death from a single infectious agent right after COVID, and its treatment is, in most cases, still based on isoniazid (INH), a compound discovered in 1952. From a combination of experimental and computational studies, three series of INH derivatives have been developed and tested [1,2]. Yet, the most promising compound series, the alkyl hydrazide series (INH-a$C_n$), which presented excellent *in silico* properties, such as membrane permeability and spontaneous IN* radical formation, seemed to be too unstable in the aqueous medium, which compromised its antitubercular activity. [2] In this work, we aim to explore the influence of halogenating the aliphatic chain to slightly deactivate the C−N bond and with that provide the well-needed stability to these compounds' series. With that in mind, we systematically added halogen atoms in different positions of the lipophilic tail of INH-a$C_4$ and estimated the IN* formation reactivity of the final derivatives using Quantum Mechanics calculations [2]. Additionally, we used Molecular Dynamics simulations to find out whether the halogenation had a negative impact on the membrane partition of the pro-drug. The fluorine and chlorine derivatives showed promising reactivities, quite similar to INH, and are expected to have higher lipophilicity than the parent compound. On the other hand, the bromine derivatives if chosen, would be quite unstable. This study allowed us to understand better the relation between the type of halogenation and the reactivity and membrane partition properties, which may be central to the development of new INH-based derivatives able to significantly decrease the scourge of multi-drug resistance.

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# P32. CO$_2$ photoreduction with tripodal Fe(II) complexes

Bento, Marcos(1); Devid, Edwin(2); Rocha, João(3); Gleeson, Michael(2); Martinho, Paulo N.(1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa.
(2) Dutch Institute for Fundamental Energy Research (DIFFER), De Zaale 20, 5612 AJ Eindhoven, The Netherlands
(3) Department of Chemistry, CICECO-Aveiro Institute of Materials, University of Aveiro, Portugal
E-mail: mambento@fc.ul.pt

CO$_2$ is one of the main greenhouse gases in the Earth's atmosphere. Currently, there is a huge interest in developing strategies to capture and convert it into chemicals with economical value.[1] Photochemical catalysis and plasma technology have been looked at as promising methods for CO$_2$ conversion operating at ambient pressure and temperature, and converting CO$_2$ into products such as CO, CH$_4$ and CH$_3$OH. [2] In CO$_2$ conversion, different types of molecular complexes or materials can be used as catalysts based in different metal centers. [3] Our work aims to synthesise new Fe(II) complexes for CO$_2$ photoreduction and plasma CO$_2$ conversion comprising homogeneous (molecular Fe(II) catalyst) and heterogeneous (MOFs functionalised with Fe(II) complexes) approaches. Here we present our preliminary results on the photoreduction of CO$_2$ to CO using visible light and the limitations in selecting a suitable photosensitiser to promote efficient electron transfer to the Fe(II) catalysts.



R = $^i$Pr, Ph, Cy or ξ–P
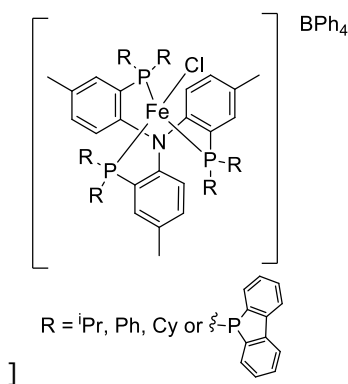
**Fig. 1.** Fe(II) complexes used as catalyst in this work.

**References**: [1] Friedlingstein P. et al. (2019) Global Carbon Budget 2019. Earth Syst. Sci. Data, 11, 1783–1838; [2] Bogaerts, A. et al. (2020) The 2020 plasma catalysis roadmap. J. Phys. D. Appl. Phys., 53, 443001; [3] Chen H. et al. (2020) Nonthermal plasma (NTP) activated metal–organic frameworks (MOFs) catalyst for catalytic CO2 hydrogenation. AIChE J., 66, 66:e16853

# P33. Measurement of $^{13}\delta$ isotope ratio in anabolic androgenic steroids by FT-ICR-MS

Oliveira, Sofia (1,2); Louro, Mariana (1,2); Sousa Silva, Marta (1,2);
Cordeiro, Carlos (1,2); Gaspar, Helena (2)

(1) FT-ICR and Structural Mass Spectrometry Laboratory,
(2) Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Portugal
E-mail: mplouro@fc.ul.pt ; fc53036@alunos.fc.ul.pt

Anabolic androgenic steroids (AAS) are synthetic derivatives that mimic the effect of testosterone. The abuse of these substances by athletes allows them to gain higher levels of muscle mass, often far beyond that obtainable by natural means. Of the 65 AAS controlled by WADA, 17 present an additional challenge concerning their identification, given that they are also biosynthesized by the human body. The distinctiveness between biosynthesized substances and those resulting from exogenous administration of endogenous AAS is extremely important regarding anti-doping control. [1] The detection of the administration of these compounds is based on the calculation of $^{13}C/^{12}C$ ($^{13}\delta$) isotope ratio. This is possible as there is a difference in the $^{13}\delta$ isotope ratio of exogenously administered endogenous AAS and naturally AAS present in the human body. Gas chromatography coupled to isotope ratio mass spectrometry with a combustion interface (GC-C-IRMS) is the technique of excellence employed in WADA accredited laboratories to determine if an atypical steroidal profile in urine is a consequence of exogenous administration of endogenous AAS. However, GC-C-IRMS is time-consuming and requires a large sample amount. The emergence of extreme resolution mass spectrometers, able to fully resolve the isotopologues of a given molecule without sample pre-treatment and in complex mixtures, such Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) hold the promise to revolutionize this analytic process. Here we show that FT-ICR-MS can be used to determine the $^{13}\delta$ isotope ratio of steroids and therefore to detect AAS misuse. [2,3] Reproducibility was tested with a calibration mixture of 4 steroids. The effects of resolution, ions excitation in the ICR cell, as well as their accumulation time and external isolation on the variation of $^{13}\delta$ isotope ratio were investigated. Preliminary results show great promise but the variability of the calculated $^{13}\delta$ isotope ratio values remains a problem currently being addressed.

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# P34. Sea urchin adhesion: one sugar to rule them all?

Ventura, Inês (1); Flammang, Patrick (2); Santos, Romana (1)

(1) MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Animal Biology Department, Faculty of Sciences, Lisbon University, 1749-016 Lisbon, Portugal
(2) Biology of Marine Organisms and Biomimetics Unit, Research Institute for Biosciences, University of Mons, Mons, Belgium
E-mail: iventura@fc.ul.pt

Commercially available bioadhesives, despite increasingly used in biomedicine and biotechnology, still face a major technical challenge: strong adhesion in wet environments. In this context, biological adhesives secreted by marine invertebrates have appealing characteristics to incorporate into new underwater biomimetic adhesives. However, successful examples inspired in marine organisms are still scarce and both industry and end-users are eagerly looking for innovative adhesives.

Biological attachment is a common feature among several marine invertebrate species. It is essential for feeding, locomotion, defense, mating, and to prevent dislodgement [1]. Echinoderms possess specialized adhesive organs, the tube feet, that consist of a mobile stem, and an adhesive disc. The disc epidermis encloses a duo-gland adhesive system, with adhesive cells producing a gluing secretion and de-adhesive cells producing a releasing secretion. Their glue is mainly composed of proteins and carbohydrates in varying proportions [1]. Glycosylation, a post-translational modification prevalent in these adhesives, has been proposed to increase conformational stability, enhance protein binding ability, and make proteins more resistant to degradation [2]. Combined approaches using recent 'omics and lectins (carbohydrate-binding proteins that bind specifically to sugars) have pinpointed adhesion related glycoproteins in flatworms, sea stars and sea urchins [1,2]. A recent study [3] performed an interspecific analysis of sea urchin adhesive composition demonstrating a high selective pressure for conservation of functional domains in large putative cohesive proteins and highlighting the importance of glycosylation in sea urchin adhesion with indications of taxonomy-related conservation of the conjugated glycans. However, only four species were considered. Therefore, to further confirm these results, we investigated 15 sea urchin species using a set of 22 lectins which were used on tube feet sections and on the proteins extracted from both the adhesive and non-adhesive component of the tube feet.

New insights regarding potential building-blocks of sea urchin adhesion are crucial to identify which proteins to mimic towards the development of new biomimetic adhesives.

**References:** [1] Flammang, P et al. (2016) Adhesive secretions in echinoderms: A Review. In Biological Adhesives, 193-222; [2] Ventura, I et al. (2023) Glycoproteins Involved in Sea Urchin Temporary Adhesion. Mar. Drugs, 21, 145; [3] Gaspar, L et al. (2021) Interspecific analysis of sea urchin adhesive composition emphasizes variability of glycans conjugated with putative adhesive proteins. Front. Mar. Sci., 8, 1931.

# P35. Development of new antimalarial therapies based on the permeation of solutes through *PfAQP*

Batista, Marta S. P. (1); Costa, Paulo J. (1); Victor, Bruno L. (1)

(1) BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016 Lisboa, Portugal
E-mail: martaspbat@fc.ul.pt

Malaria is one of the largest public health problems. Although most variants are successfully treated with the existing antimalarial drugs, this disease is still responsible for a large number of global deaths. Severe malaria in humans is mostly caused by infection with Plasmodium (P.) falciparum whose complications include severe anemia, end-organ damage, pulmonary complications, and hypoglycemia [1]. The development of hybrid antimalarial agents has been pursued as a promising strategy to tackle resistant parasite strains, eliminating the actively-infecting P. falciparum organisms in human red blood cells, and also the replicative and dormant liver forms of the parasite [2]. The aquaporin of P. falciparum (PfAQP) is a water and glycerol membrane protein channel, allowing the permeation of these molecules from the host to the parasite. The fast reproduction of P. falciparum in the host red blood cells requires massive biogenesis, in which glycerol is incorporated into the lipids of newly synthesized parasite membranes [3]. Therefore, PfAQP is seen as a promising therapeutic target for the development of new antimalarial therapies.

In this communication, we will present a recently developed bioinformatics approach focused on the identification of PfAQP structural features that regulate the permeation of water, glycerol, erythritol, and xylitol through its pores, to boost the development of a hybrid therapeutical agent that either blocks or transports currently available antimalarial drugs to P. falciparum medium. By using methods such as Molecular Dynamics, Umbrella Sampling, and Potential of Mean Force calculations we gathered relevant permeation-related structural information, which will hopefully allow the identification and development of new antimalarial hybrid drugs.

**References:** [1] Phillips, M.A. et al. (2017) Malaria. Nat Rev Dis Primers 3, 17050; [2] Capela, R. et al. (2011) Design and Evaluation of Primaquine-Artemisinin Hybrids as a Multistage Antimalarial Strategy. Antimicrob Agents Chemother, 55, 4698–4706; [3] Dean, P. et al. (2014) Transport proteins of parasitic protists and their role in nutrient salvage. Front Plant Sci, 5, 153.

# P36. Impact of non-enzymatic post-translational modification on fatty acid oxidation enzymes

Domingos, R. M. (1), Ribeiro, J. V. (1), Gomes, C. M. (1), Henriques, B. J. (1)

(1) BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
E-mail: ruymdomingos@gmail.com

Metabolic regulation encompasses a complex interplay of genomic, proteomic, and metabolomic adjustments within cells. Recent advancements have revealed the widespread presence of a particular group of non-enzymatic post-translational modifications (PTMs) known as acylations, such as glutarylation and succinylation in mitochondrial enzymes [1]. Acylations appear to play pivotal roles in regulating mitochondrial metabolism through sirtuin-mediated pathways.

The extent of acylations is closely associated with the accumulation of intermediate metabolites such as succinyl-CoA, and glutaryl-CoA that occur under certain conditions such as fasting, caloric restriction or in several metabolic disorders, creating a unique scenario for anomalous protein acylation [2]. Recently, we showed that when glutaryl-CoA production is stimulated by lysine catabolism, glutarylation on the lysine oxidation pathway enzyme glutaryl-CoA dehydrogenase (GCDH) increases [3]. Further, we demonstrated the ability of sirtuin5 (Sirt5) to deglutarylate GCDH regulating is function.

Following an emerging idea that enzymes from pathways that handle these metabolites are more susceptible to protein acylation, here we combine biochemical and biophysical methods to evaluate acylation effects on Medium Chain Acyl-CoA Dehydrogenase (MCAD) enzyme structure and function.

We showed that purified MCAD is easily succinylated and glutarylated. Focusing on succinylation, we show that modified MCAD presents an overall fold and stability similar to the unmodified control, however the enzyme activity is increased. Preliminary assays also indicate that sirtuin5 incubation may partially recover normal enzyme function.

**References:** [1] Wagner, G.R. et al. (2017) A Class of Reactive Acyl-CoA Species Reveals the Non-enzymatic Origins of Protein Acylation. Cell Metab, 25, 823-837. [2] Pougovkina, O. et al. (2014) Aberrant protein acylation is a common observation in inborn errors of acyl-CoA metabolism. J Inherit Metab Dis, 37, 709-14. [3] Bhatt, D.P., et al. (2022) Deglutarylation of glutaryl-CoA dehydrogenase by deacylating enzyme SIRT5 promotes lysine oxidation in mice. J Biol Chem, 298, 101723.

# P37. A new dual role for the cytoskeleton modulator INF2 in the regulation of CFTR

Ferreira, João (1); Farinha, Carlos (1)

(1) BioISI-Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Campo Grande, 1749-016 Lisboa
E-mail: jfsferreira@ciencias.ulisboa.pt

Background: Old and new data has made increasingly clear that CFTR is at a crossing between the cytoskeleton and signaling pathways, especially cAMP signaling. Fine tuning of CFTR regulation requires integrity of correct cytoskeletal organization, as the cytoskeleton is responsible for the scaffolding that stabilizes CFTR at the plasma membrane (PM) and brings several interacting proteins to CFTR's proximity, among which cAMP sensors, such as PKA and EPAC1, have a prominent role [1]. Regulation of CFTR also occurs with recruitment of several actin cytoskeleton dynamics regulators to CFTR's proximity, namely INF2. INF2 is an unusual member of the formin family because of its unique ability to accelerate actin filament depolymerization, adding to the nucleation and elongation activities common to all formins and also its unique ability to associate reversibly with the cytosolic leaflet of the endoplasmic reticulum (ER). INF2 has been reported as a negative regulator of CFTR PM stability [2]. The main goal of the present work was to characterize the dual role of INF2 in the regulation of CFTR both at the PM and ER.

Methods: We used Cystic Fibrosis (CF) bronchial epithelial cells expressing wild-type (WT)- or p.Phe508del-CFTR and analyzed them using Western blot and cycloheximide chase assay.

Results: Western blot results show that INF2 knockdown (KD) promotes an increase in mature WT-CFTR levels, even after treatment with the corrector combination VX-661 + VX-445. For p.Phe508del-CFTR, results show that INF2 KD improves rescue by VX-661 and by VX-661 + VX-445, independently of EPAC1 activation. In both experiments it is also observed that INF2 KD promotes a strong stabilizing effect on immature CFTR levels, unravelling a role of INF2 in the ER in early CFTR traffic that has not been characterized. In a cycloheximide-chase assay, it was identified that INF2 KD decreases rescued p.Phe508del-CFTR turnover, with an increase in protein half-life. This suggests that the stabilizing effect of INF2 KD on rescued p.Phe508del-CFTR at the PM is caused by a decrease in degradation, possibly through a decrease in endocytosis or an increase in recycling. These results indicate that INF2 has a dual role in the regulation of CFTR, both at the PM and ER, and it should be considered as a novel potential target for modulation to develop new combinatorial therapies for CF.

Conclusions: These observations constitute an important characterization of how an actin cytoskeleton dynamics regulator regulates CFTR, exploring the crosstalk between cAMP signaling pathways and the cytoskeleton to affect CFTR modulation, and possibly CF handling.

**References:** [1] Lobo, M et al. (2016) EPAC1 activation by cAMP stabilizes CFTR at the membrane by promoting its interaction with NHERF1. J Cell Sci., 129, 2599–2612; [2] Santos, J et al. (2020) Cytoskeleton regulators CAPZA2 and INF2 associate with CFTR to control its plasma membrane levels under EPAC1 activation. Biochem J., 477, 2561-2580.

# P38. *E(xtraterrestris). Coli* Adapting genome-scale metabolic models to non-standard thermodynamical constraints

Monteiro, Lucas (1); Pinto, Francisco (2); Moitinho, André (3)

(1) Universidade de Lisboa, Departamento de Informática, FCUL
(2) Universidade de Lisboa, BioISI BioSystems and Integrative Sciences Institute, FCUL
(3) Universidade de Lisboa, CENTRA Center for Astrophysics and Gravitation, FCUL
E-mail: fc52849@alunos.fc.ul.pt

Since the dawn of mankind, humans have looked to the sky in awe, wondering what hidden worlds lurk through the cosmos. Our imagination led us to take steps on the moon, and now, with a new space race beginning [1], the possibility of extraterrestrial settlements arises. This raises the question: how might our presence disrupt alien environments? Could humans act as carriers of potential contaminants?

Equipment or humans themselves could carry organisms that adapt and thrive in hostile environments [2]. Genome-scale metabolic models (GEMs) can be used to simulate the behavior of simple organisms [3], but these models are typically available under standard conditions (NTP).

In this study, we adapt genome-scale metabolic models to non-standard temperatures using thermochemistry group contribution estimations and temperature corrections. Additionally, we employ kinetic modeling for parameter coherence and calculate new metabolic flux predictions. To validate our approach, we test the method using an Escherichia coli core GEM and predict its survivability under a wide range of temperatures and various known planetary conditions.

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# P39. Sterol and sphingolipid segregation into two major types of domains in the yeast plasma membrane

Guimarães, Mafalda (1); Bento-Oliveira, Andreia (1); Gomes, Margarida (1); Silveira, Vasco (1); Marquês, Joaquim T. (1); de Almeida, Rodrigo F. M. (1)

(1) Centro de Química Estrutural, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Portugal
E-mail: fc54718@alunos.fc.ul.pt

The Yeast plasma membrane (PM) has been widely studied and currently the presence of two major membrane compartments is acknowledged, one occupied by Can1p (MCC) and enriched in ergosterol and another occupied by Pma1p (MCP) and enriched in sphingolipids [1]. The presence of highly rigid sphingolipid-enriched domains (SLEDs) in the PM of yeast under physiological conditions [2] is a major difference from the mammalian PM. The studies of the *S. Cerevisiae* PM will contribute to identify the main differences between fungal and mammalian membranes and better understand the action of antifungals, which will be crucial for the development of more effective antifungal therapeutics.

With the goal of studying the biophysical properties and organization of the yeast PM, the PM of *S. cerevisiae* yeast cells was isolated through differential centrifugation and labeled with different fluorescence membrane probes: DPH, di-8-ANEPPS and *t*-PnA. DPH is a good reporter of the global membrane order; di-8-ANEPPS is particularly sensitive to domains enriched with sterols reporting on properties such as the dipole potential of the membrane, whereas *t*-PnA is particularly suitable to study gel domains, such as SLEDS. Wild-type *(wt)*, *scs7Δ* and *erg6Δ* mutant strains were studied. The use of *scs7Δ* cells that do not express the enzyme required for the 2-hydroxylation of the sphingolipid long chain fatty acid [1,2] will provide insight into the role of sphingolipid hydroxylation in the formation and stabilization of SLEDs. The *erg6Δ* does not produce ergosterol and accumulates zymosterol and cholesta-5,7,24-trienol, instead.

The fluorescence anisotropy values obtained, when using both DPH and di-8-ANEPPS, were not significantly different for the three strains, which suggest that the membrane global order was the same despite the different lipid profiles of the strains used. The values of dipole potential were not significantly different between *wt* and *scs7Δ* cells. However, there were differences between *wt* and *erg6Δ* mutant strains. This indicates that the dipole potential of yeast PM is strongly influenced by the sterol but not the sphingolipid profile. Regarding *t*-PnA, the fluorescence lifetimes show that while gel domains present the same rigidity in *wt* and *erg6Δ* cells, they are more compact in *scs7Δ* cells. These results, obtained with isolated yeast PM, comprise a crucial piece of evidence for the strong segregation of two major types of lipid domains in the fungal PM, sphingolipid-enriched and sterol-enriched domains.

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# P40. Enhancement of mRNA for improved vaccine production

Ji, Vanessa X. (1); Azevedo, Ana M. (1); Silva-Santos, A. Rita (1)

(1) Department of Bioengineering, iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
E-mail: vanessa.x.ji@tecnico.ulisboa.pt

Since the development of the first vaccine, multiple delivery systems have emerged, rendering vaccination one of the most successful and impactful public health advancements in history. The unprecedented Covid-19 pandemic brought the freshly developed messenger RNA (mRNA)-based platform into the limelight. This technology allowed the rapid development of an authorized vaccine within a remarkably brief duration of less than one year. Despite notable advancements in the field, establishing robust and economically viable manufacturing processes that consistently yield high-quality mRNA vaccines remains imperative.

This project aims to enhance the mRNA manufacturing process, specifically upstream processing, focusing on optimizing the efficiency of in vitro transcription (IVT) reactions by modifying the mRNA sequence and structure. During IVT, the formation of double-stranded RNA (dsRNA) is critical, as its presence may complicate downstream processes. It can lead to the activation of dsRNA-specific cellular response pathways, reducing overall translation efficiency. Therefore, it is hypothesized that controlling the generation of by-products, including dsRNA, through strategic mRNA sequence optimization can enhance the overall efficiency and output of an already optimized IVT process [1].

The synthetic mRNA template used in this research comprises a 3' poly(A) tail and an enhanced green fluorescent protein (eGFP) gene flanked by a 5'- and 3'- untranslated regions (UTRs) [1]. These regions' adjustment is pivotal as it directly impacts translation efficiency, a determinant of protein synthesis rates. To potentially optimize this influence, an analysis of seven unique 5´-UTRs is being conducted [2]. The quantification and assessment of both mRNA and dsRNA are executed through reverse-phase high-performance liquid chromatography (RP-HPLC) and gel electrophoresis, while the eGFP serves as a reporter, enabling the assessment of translation efficiencies through fluorescence spectroscopy.

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# P41. Scalling up ssDNA scaffold purification for DNA-origami nanostructures assembly

Mendes, Joana (1); Prazeres, D.M.F. (1); Silva-Santos, A. Rita (1)

(1) iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal
E-mail: joana.silva.mendes@tecnico.ulisboa.pt

DNA nanostructures are often assembled using the 'scaffolded DNA origami' strategy, in which a long (~103-104 bp) single stranded DNA (ssDNA) is folded into a designed nanostructure with the aid of many shorter staple strands (oligonucleotides). The most frequently used DNA scaffold is the single-stranded 7249-nucleotide circular M13mp18 genome [1].

However, one crucial step in ssDNA scaffold preparation for DNA-origami nanostructures assembly is purification. With this project we aim to set up scalable production and purification methods to obtain suitable ssDNA scaffolds from the DNA of the M13 bacteriophage.

M13 phage was produced in *E. coli* cultures/bioreactor and then, the M13mp18 genome was purified and chemically lysed with 200 mM NaOH/1 % SDS to release the ssDNA, followed by neutralization with acetate. Afterwards, purification of the M13mp18 ssDNA by means of ultrafiltration and/or anion-exchange chromatography was evaluated, considering different filtration cut-offs and chromatographic resins.

Final ssDNA product purity was determined by HPLC and agarose gel electrophoresis. Presence of impurities like host cell proteins and host DNA is evaluated using BCA and PicoGreen. The purified ssDNA was used as scaffold for the folding of an asymmetric DNA-origami structure with the suitable set of staple strands.

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# P42. *Saccharomyces cerevisiae* Plasma Membrane: Fluorescence Insights into Membrane Heterogeneity

Leitão, Helena (1); Trigo Marquês, Joaquim (1); F. M. de Almeida, Rodrigo (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
E-mail: fc57776@alunos.fc.ul.pt

For the last few decades, pathogenic fungi have been considered a major concern due to the rise in cases of infection and increasing resistance to current available antifungal therapeutics [1]. Recent studies show that the highly rigid sphingolipid-enriched domains (SLEDs) found at the plasma membrane (PM) of fungi, such as *Saccharomyces cerevisiae*, appear to be absent in mammalian cells, which qualifies as potential targets for antifungal drug therapies [2].

The main purpose of this project is to clarify the role that different biophysical properties of the lipid membranes of yeast *S. cerevisiae* have in antifungal resistance. Specifically, we aim at finding alternative methods for labelling SLEDs, as the only currently available method poses practical limitations.

In this study, we labelled the yeast membrane with 1,6-diphenyl-1,3,5-hexatriene (DPH) and two DPH derivatives, namely the cationic trimethylammonium derivative (TMA-DPH) and the anionic propanoic acid derivative (PA-DPH), which have the potential to label different regions of the yeast PM as a consequence of their charge. For example, it is reported that TMA-DPH is retained in the outer leaflet of the PM due to its positive charge [3]. Moreover, we hypothesize that it interacts preferentially with SLEDs due to the negative charge of complex SL in yeast (inositolphosphorylceramide based). On another hand, DPH-PA is being used for the biophysical characterization of yeast PM for the first time and should behave distinctly from TMA-DPH.

Cell viability was assessed in yeast cells, and it was not affected by any of the fluorescent probes (> 99.0 %). In addition, the kinetics of steady-state fluorescence anisotropy of the probes added to yeast cell suspension strongly suggests that DPH distributes evenly among the PM and inner membranes, whereas its derivatives seem to concentrate at the PM, a conclusion that is corroborated by fluorescence microscopy imaging. Moreover, it seems that the derivatives label different membrane domains at short times. To further understand the nature of these domains, this study will comprise fluorescence lifetime measurements and the use of deletion mutant yeast cells in sterol and sphingolipid biosynthetic pathways, in order to clarify the membrane biophysical properties that may for some of these deletions increased resistance to antifungal agents.

**References:** [1] Brown, G. D. et al. (2012). Hidden Killers: Human Fungal Infections. Sci Transl Med, 4, 165rv13; [2] Santos, F. C. et al. (2020). Sphingolipid-enriched domains in fungi. FEBS Lett, 594: 3698-3718; [3] Chazotte, B. (2011). Labeling the Plasma Membrane with TMA-DPH. Cold Spring Harb Protoc, 5.

# P43. Developing Synthetic Adhesive Proteins Inspired by Sea Urchin Nectin Structural Domains

Santos, Mariana R. (1); Ventura, Inês (2); Gomes, Cláudio M. (1); Santos, Romana (2); Henriques, Bárbara J. (1)

(1) BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
(2) MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
E-mail: fc52690@alunos.fc.ul.pt

Nowadays there is a great need for biological adhesives that are effective in wet/humid environments for biomedicine and biotechnology applications (for example, to be used in surgical adhesives or to promote cellular adhesion for *in vitro* cultures). Marine invertebrates produce secretions with remarkable adhesive properties, which can inspire the development of new biomimetic adhesives for these applications.

In the last decade, studies on the sea urchin *Paracentrotus lividus* identified nectin as an important adhesive protein present in its adhesive organs (tube feet) and adhesive secretions [1]. Nectin has six galactose-binding discoidin-like (DS) domains, which are thought to be important for its adhesive function [1-3]. Since nectin is a fairly large protein (>100 kDa), it would be very laborious to produce and purify it in an industrial scale.

The main goal of my project is to discover one or more combinations of DS domains that will simultaneously confer adhesive properties and adequate stability comparable to the full-length protein, as well as optimized protocols for expression and purification of the identified targets.

To achieve our goal, we have investigated six different constructs of nectin domains for bacterial recombinant protein expression. At the moment, we have established expression and purification protocols for four of them and we are in the process of characterizing them in respect to structure and conformational stability, using techniques like circular dichroism (CD), fluorescence spectroscopy and differential scanning fluorometry (DSF).

**References:** [1] Santos, R. et al. (2013) Mapping sea urchins tube feet proteome - A unique hydraulic mechano-sensory adhesive organ. J Proteomics, 79, 100-113; [2] Pjeta, R et al. (2020) Integrative Transcriptome and Proteome Analysis of the Tube Foot and Adhesive Secretions of the Sea Urchin *Paracentrotus lividus*. Int J Mol Sci, 21, 946; [3] Costa, C et al. (2010) Phylogenetic analysis and homology modelling of *Paracentrotus lividus nectin*. Mol Divers, 14, 653-665.

# P44. Development of HIV-dependent gene expression kill-switches for gene therapy applications.

Moraes, Filipa (1); Fernandes, Carolina (1); Cebolla, Lara (1); Scouarnec, Jade (1); Othman, Eman (1); Marques, Tânia (1); Gama-Carvalho, Margarida (1).

(1) BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisboa, Portugal
E-mail: filipatrigomoraes@gmail.com

In HIV-infected individuals, the virus remains latent inside reservoir cells, which have the potential to reactivate and transcribe integrated provirus. This remains a persistent impediment to curing the disease, as current therapies to control HIV do not mitigate this risk. The advent of genome editing approaches based on the CRISPR-Cas9 system has opened new opportunities for targeting integrated proviral DNA. However, this strategy raises significant concerns regarding the possibility of off-target effects. Furthermore, the Cas9 protein has been shown to elicit unwarranted immune responses, imposing a barrier to its clinical application. We are developing a strategy to exclusively target the delivery of active dCas9/sgRNA complexes to reservoir cells harboring proviral DNA, taking advantage of viral regulatory proteins and highly cell type-specific miRNA signature profiles. This profile combines both positive and negative regulatory elements to tightly control the system's cell-type specificity, with negative elements being selected from a library of randomized arrays of three to five distinct MRE sequences. The full platform can be used to achieve specific elimination of the target cell population through controlled viral re-activation inducing cellular toxicity. This miRNA-regulated system is being developed in a flexible way supporting its adaption to other scenarios where the identification and elimination of cells presenting specific genomic sequence elements is of interest.
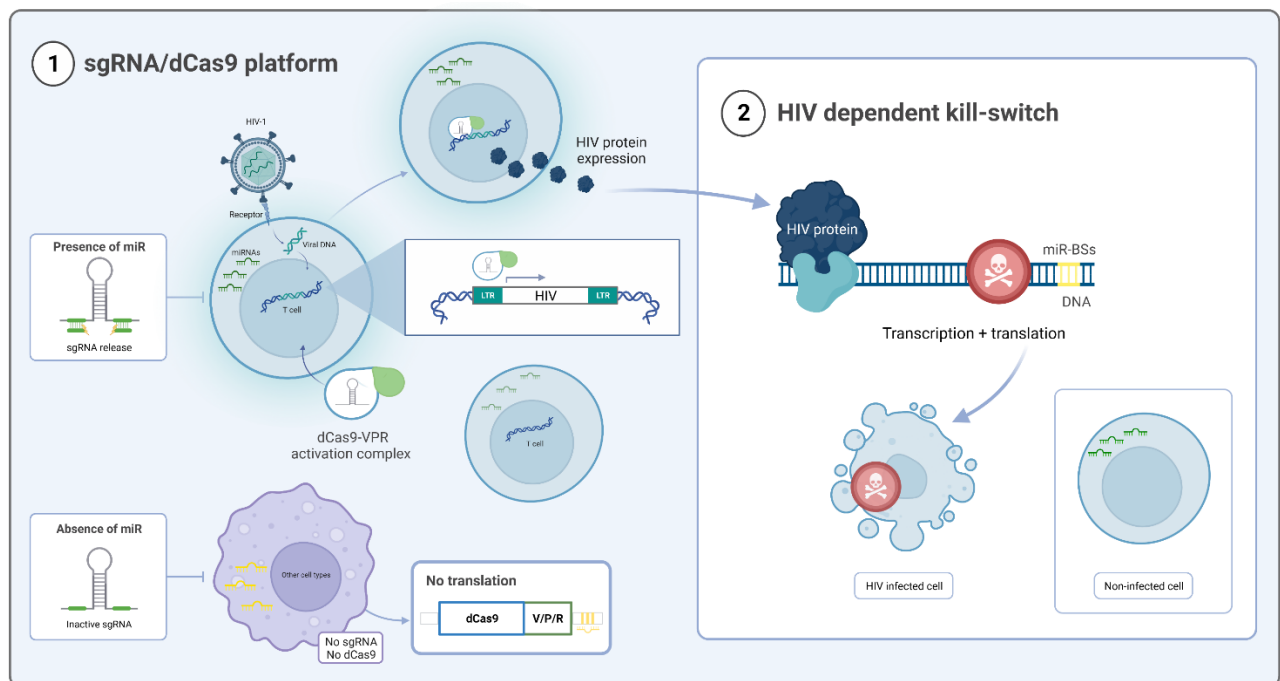
**Fig. 1.** Schematic representation of the full HIV-killing system, which includes 1) the small guide RNA and dCas9 platform and 2) the HIV-1 dependent switches.

# P45. Single-Domain Antibodies as Tools for Controlling Tau Aggregation in Neurodegenerative Diseases

Simões, Margarida (1,2); Cristóvão, Joana S. (1,2); Pardon, Els (3), Steyaert, Jan (3); Gomes, Cláudio M. (1,2)

(1) BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Portugal
(2) Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal
(3) VIB-VUB Center for Structural Biology, Brussels
E-mail: margarida.csimoes@hotmail.com

Aggregation of amyloid-β (Aβ) and Tau protein into higher-order aggregates is a hallmark of Alzheimer's disease (AD) [1]. The accumulation of these pathological aggregates in the aged brain promotes neuroinflammation processes responsible for the secretion of alarmins, including S100B protein. S100B is a multifunctional protein that exerts both neurotrophic and deleterious effects, associated respectively with early and late stages of disease [2]. Recently, the host laboratory discovered a novel S100B role as a protective anti-aggregation chaperone over Aβ and Tau [3,4]. S100B dual function – deleterious and beneficial – makes it an amenable drug target. To modulate S100B chaperone activity and potentiate its anti-aggregation behavior, we developed a library of more than 25 anti-S100B nanobodies. Here, we report that using ThT-monitored aggregation kinetics, several nanobodies potentiated S100B inhibitory effect over K18 (Tau$_{244-372}$), possibly by locking S100B in a more suitable conformation to bind K18. Surprisingly, control experiments revealed that nanobodies alone significantly inhibit K18 aggregation even at sub-stoichiometric ratios. This observation is discussed in the context of possible heterotypic interactions between the nanobody and Tau/K18. Further, mechanistic analysis demonstrates that different nanobodies target multiple steps of K18 fibrillation. These findings uncover the therapeutic potential of anti-S100B nanobodies, which can be used as modulators of K18 aggregation or activators of S100B chaperone activity.

**References:** [1] Lane, C.A. et al. (2018) Alzheimer's disease. Eur J Neurol, 25, 59-70. [2] Cristóvão, J.S. et al. (2019) S100 Proteins in Alzheimer's Disease. Front Neurosci, 13, 463. [3] Cristóvão, J.S. et al. (2018) The neuronal S100B protein is a calcium-tuned suppressor of amyloid-β aggregation. Sci. Adv, 4, eaaq1702. [4] Moreira, G.G. et al. (2021) Dynamic interaction and Ca(2+)-binding modulate the holdase-type chaperone activity of S100B preventing tau aggregation and seeding. Nat Commun, 12, 6292.

# P46. Identification of genes that restore p.Phe508del-CFTR traffic in cystic fibrosis models through GRK5 inhibition

Neugebauer, Daniela (1); Cachatra, Vasco (2); Moiteiro, Cristina (2); Amaral, Margarida D. (1); Botelho, Hugo M. (1)

(1) Biosystems & Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisboa, Lisboa, Portugal
(2) Centro de Química Estrutural, Institute of Molecular Sciences, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisboa. Lisboa, Portugal
E-mail: dneugebauer@fc.ul.pt

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in Caucasians and is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR protein, which normally operates as a chloride and bicarbonate channel in the plasma membrane (PM) of epithelial cells, exhibits distinct dysfunctions depending on the mutation. The most prevalent CF-causing variant, p.Phe508del, causes CFTR misfolding, which results in CFTR retention by the endoplasmic reticulum (ER) quality control and early degradation, thus preventing successful traffic of CFTR to the PM. Small-molecule drugs, termed CFTR modulators, are in the clinic and rescue p.Phe508del-CFTR function or traffic. While significantly improving life quality of patients, current modulators face high medication's costs and still provide considerable scope for improvement of effectiveness. Identification of CFTR traffic regulators may pave the way to new therapeutical avenues that aim at using these protein factors as drug targets. In a previous study [1], our group identified G protein-coupled receptor kinase 5 (GRK5) as a novel p.Phe508del-CFTR regulator whose inhibition restores p.Phe508del-CFTR PM trafficking. Rescue of p.Phe508del-CFTR was additive to current CFTR modulators, suggesting a way to maximize the efficacy of CF pharmacotherapy. However, the composition of the GRK5-CFTR signaling pathway remains unknown. In this project, we aim at elucidating this pathway by identifying genes that couple GRK5 inhibition to p.Phe508del-CFTR relocation in CF cellular models.

To identify p.Phe508del-CFTR traffic regulators which are dependent on the GRK5 activation status we quantified p.Phe508del-CFTR PM levels using an established fluorescence microscopy assay [2] while knocking-down 227 CFTR traffic regulators we previously described using siRNA. The assay was performed with and without the addition of the specific GRK5 inhibitor (R,Z)-3-((4-(2-bromoacetamido)-3,5-dimethyl-1H-pyrrol-2-yl)-methylene)-2-oxo-N-(1-phenylethyl)indoline-5-carboxamide (9g) [3]. We will present preliminary results on GRK5-dependent CFTR traffic regulators and discuss their potential biomedical relevance for CF therapy. We will also present perspectives for this project, including identification of GRK5 interactors via immunoprecipitation.

**References:** [1] Botelho, HM et al. (2023, under revision); [2] Botelho, HM et al. (2015) Sci Rep, 5, 1-8; [3] Rowlands, RA et al. (2021) J Med Chem, 64, 566-585.

# P47. Investigating the pH-Dependent Structure of Cationic Peptide Dendrimers and Their Potential as siRNA Vectors: An In-Silico Study

Rodrigues, Filipe E. P. (1); Darbre, Tamis (2); Machuqueiro, Miguel (1)

(1) BioISI: Biosystems and Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) Department of Chemistry, Biochemistry and Pharmacy, Bern University, 3012 Bern, Switzerland
E-mail: ferodrigues@ciencias.ulisboa.pt

Peptide dendrimers are molecular structures composed of amino acid residues, exhibiting a symmetrical, tree-like shape with a well-defined and uniform topology. These dendrimers interact with various biological targets, such as nucleic acids and biological membranes. They possess antimicrobial properties and serve as effective carriers for nucleic acids [1]. Recently, researchers have explored the potential of peptide dendrimers as vectors for siRNA molecules [2]. As a result, specific dendrimers, namely MH13, MH18, and MH47, have been identified. These dendrimers consist exclusively of lysine and leucine residues and contain hydrophobic cores composed of two palmitoyl chains or a leucine tetrapeptide. The presence of hydrophobic cores enhances their ability to interact with cellular membranes and facilitates internalization through endocytosis. Moreover, their different protonation states at physiological and low pH levels play a crucial role in interacting with negatively charged nucleic acids and escaping endosomal entrapment. Notably, certain mutations in MH18, where L-amino acids are substituted with their D-counterparts, negatively affect binding and activity. Only the homochiral D-dendrimer exhibits comparable activity to the L-dendrimer. Despite numerous experimental results, our understanding of the overall molecular mechanisms and the factors governing specific properties of these structures remains limited [3].

In this study, we present our findings on the application of our advanced CpHMD methodology to investigate the pH-dependent conformational space of MH13, MH18, MH47, and other variants composed of different combinations of L and D-amino acids. We assess their pH titration behavior and perform structural characterizations, including measuring the radius of gyration and permuted root mean square deviation (RMSD). These investigations are conducted both in solution and using a lipid membrane model to evaluate the impact of the membrane on the dendrimers' conformational space and protonation behavior. Collectively, these results provide valuable insights for experimentalists, aiding in the interpretation of their data and fostering a better understanding of the molecular mechanisms underlying the dendrimers' cell internalization properties. Additionally, this knowledge can inform the design of new and improved dendrimer sequences.

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# P48. Potential of Radiation Therapy in the Disruption of Amyloid Deposits

Coelho, Carina Marques (1,2,3); Pereira, Lia (1,2,3); Teubig, Pamela (1,2); Murtinheira, Fernanda (1,3); Santos, Pedro (4); Mendes, Filipa (4); Viñals, Sílvia (5,6); Galaviz, Daniel (1,2); Herrera, Federico (1,3)

(1) Faculdade de Ciências, Universidade de Lisboa (FCUL)
(2) Laboratório de Instrumentação e Física Experimental de Partículas (LIP)
(3) BioISI - Instituto de Biosistemas e Ciências Integrativas, FCUL
(4) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico
(5) Centro de Micro-Análisis de Materiales (CMAM), Universidad Autónoma de Madrid
(6) Universidad Complutense de Madrid
E-mail: cmcoelho@lip.pt; fherrera@fc.ul.pt

Amyloid deposits are a buildup of an abnormal protein (amyloid) in organs and tissues, stopping them to working properly, and eventually leading to organ dysfunction and degeneration. Radiotherapy (RT) is a well-established medical modality delivered to more than 50% of cancer patients, but a generally ignored fact is that it has been successfully used to treat extra-cranial amyloidosis, and current evidence indicates that it could be a promising treatment for amyloid-associated neurodegenerative disorders [1]. Proton Therapy has substantial clinical advantages over conventional RT based on photons or electrons, including lower toxicity to healthy surrounding tissues. This modality is currently tested in cancer settings, but it is largely untested in the context of amyloidosis and neurodegenerative disorders. Our goal is to evaluate the capability and mechanisms of different RT modalities to disrupt or diminish the formation of toxic protein amyloids associated with neurodegenerative disorders, bringing together fundamental nuclear physics and biochemistry. Gamma-irradiations of cell lines expressing neurodegenerative disease-associated proteins, indicated a decrease in the expression and aggregation of the pathological proteins, which was proportional to the applied dose. These results have encouraged a proton irradiation experiment to established cell lines at the implantation beam line of the CMAM laboratory, and for that some dosimetric measurements were performed and an online dosimetric system was established. Our scope is to lay the groundwork for the application of PT beyond cancer, multiplying the versatility of new proton therapy facilities, and modifying the development of currently incurable neurodegenerative disorders.

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# P49. Asymmetric post-translational modifications as a new regulatory mechanism in the self-associating transcription factor STAT3

Costa, André (1); Diallo, Mickael (1); Murtinheira, Fernanda (1); Alves, Daniela (1); Pinto, Francisco (1); Torres, Vukosava (1); Rodrigues, Mario S. (1); Herrera, Federico (1)

(1) Faculty of Sciences of the University of Lisbon, BioISI - Biosystems & Integrative Sciences Institute
E-mail: aadcosta@fc.ul.pt; fherrera@fc.ul.pt

Signal Transducer and Activation of Transcription 3 (STAT3) is a transcription factor that plays a pivotal role in cellular processes, including growth, differentiation and survival, by responding to a diverse range of growth factors and cytokines. Upon canonical tyrosine phosphorylation, STAT3 homodimers accumulate in the nucleus and regulate the expression of numerous genes related to these cellular processes. However, STAT3 undergoes more than 80 post-translational modifications (PTMs), and their stoichiometry and specific functions remain largely unexplored. We present a multidisciplinary investigation exploring the potential impact of asymmetric PTMs on the behavior and functionality of STAT3 homodimers (i.e. PTMs that selectively occur in a single monomer). Using bioimaging methodologies, we are able to visualize and analyze the dynamic localization and interactions of modified STAT3 homodimers within live cells. To examine the cellular distribution of STAT3 dimers in STAT3KO Hela living cells, we have developed a Venus-STAT3 bimolecular fluorescence complementation (BiFC) assay. We are investigating the impact of dimers carrying asymmetric inactivating mutations on residues that are susceptible to PTMs, which may influence the cellular distribution of STAT3 in quiescent cells. Our findings indicate that these asymmetric mutations also affect the nuclear translocation of STAT3 following induction with Leukemia inhibitory factor (LIF). To gain a deeper understanding of the impact of asymmetric PTMs, we are employing a combination of proteomic and bioinformatics techniques. We have effectively identified exclusive protein among different conditions or experimental groups. This integrated approach allows us to explore the consequences of these modifications and provides valuable insights into their functional implications. Our aim is to identify the specific PTMs that occur in a monomer-specific manner. Our research will advance our understanding of STAT3 regulation, which is relevant to a wide spectrum of human pathologies, such as hyper-IgE syndrome, cancer, spinal cord injury, stroke or neurodegenerative disorders.

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# P50. Biophysical Analysis of Lipid Domains in Mammalian and Fungi Model Membranes

Cuhadar, Elif Dicle (1); Bento-Oliveira, A. (1); Alena Khmelinskaia (1); Marinho, H. Susana (1); Marquês, Joaquim T. (1); de Almeida, Rodrigo F.M. (1)

(1) Centro de Química e Bioquímica, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciêncas, Universidade de Lisboa, Ed.C8, Campo Grande, 1749-016 Lisboa, Portugal
E-mail: elif_dicle@icloud.com

Lipid membranes play a crucial role in maintaining the structural integrity and functionality of cells. Sphingolipids, have emerged as a key players in modulating membrane organization. In fact sphingolipid-enriched domains (SLEDs) in a gel phase are present under physiological conditions in the plasma membrane of fungi, but not in that of mammalian. Phytoceramides and ceramides, which differ in their hydroxylation levels, are the major backbone of complex sphingolipids in fungi and mammals, respectively. On the other hand, while ergosterol is the main sterol in fungi, cholesterol is the major sterol in mammalian membranes [1].

In this study, we investigated the mutual role of sphingolipid hydroxylation pattern and sterol type in membrane biophysical properties and organization in the presence of a representative glycerophospholipid, palmitoyloleoylphosphatidylcholine (POPC). Three-component (POPC/sphingolipid/sterol) lipid systems were studied. Ceramide and phytoceramide were the sphingolipids used and ergosterol, cholesterol and their last common biosynthetic precursor, zymosterol, were the sterols assayed. Lipid bilayers were labeled with two fluorescent membrane probes: *trans*-parinaric acid (*t*-PnA), which is particularly suitable to study gel domains and 4-(2-(6-(dibutlylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-pyridinium(di-4-ANEPPS), a probe that preferentially partitions into domains enriched in sterols. Steady-state and time-resolved fluorescence spectroscopy were employed. In most systems, the expected behavior, i.e., typical solubilization of sphingolipid-enriched gel domains at high sterol mole fractions was observed. The most striking result shows an increase in *t*-PnA fluorescence lifetime for the POPC/ceramide/ergosterol systems as the sterol molar fraction increases, a behavior unique to this system. This result may suggest that in this system, sterol and sphingolipid mutually segregate. Overall, the results obtained highlight the role of sphingolipid hydroxylation in modulating sphingolipid/sterol interactions, which may explain not only why cholesterol is the major sterol in mammals and ergosterol in fungi, but also the presence of SLEDs in the gel phase in fungal plasma membrane.

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# P51. Fabrication of Cell-Loaded Delivery Systems for Regenerative Medicine Applications Using Microfluidics

Patrício, Ana (1,2,3); Duarte, Sofia (1,2); Silverio, Vania (2,3,4); Fonte, Pedro (1,2,5,6)

(1) iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Portugal
(2) Associate Laboratory i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Portugal
(3) Institute of Systems and Computer Engineering for Microsystems and Nanotechnologies, INESC MN, Portugal
(4) Department of Physics, Instituto Superior Técnico, Universidade de Lisboa, Portugal
(5) Center of Marine Sciences (CCMAR), Universidade do Algarve, Portugal
(6) Department of Chemistry and Pharmacy, Faculty of Sciences and Technology, Universidade do Algarve, Portugal
E-mail: prfonte@ualg.pt

Chronic wounds are a significant public health challenge, with significant economic and quality of life implications. Medicare data from 2018 showed that chronic and non-healing wounds affected approximately 8.2 million beneficiaries, resulting in projected costs ranging from $28.1 billion to $96.8 billion. [1] Management of infections, particularly in surgical wounds and diabetic ulcers, accounted for a significant portion of these costs. Regenerative medicine offers promising solutions for effective wound healing, and stem cell therapy has demonstrated safety and efficacy in both preclinical and clinical studies. However, stem cell delivery remains a major challenge due to issues such as difficulties in tissue targeting, high shear stress and low cell survival. [2] To overcome these challenges, cell-loaded delivery systems such as alginate microparticles have demonstrated the ability to support cell viability, functionality, and protection against environmental stressors. [3] The aim of this work is to develop a microfluidic platform for the production of mesenchymal stem cells and insulin-loaded microparticles. Spherical and stable unloaded microparticles were successfully obtained at different lipid and aqueous flows using the fabricated microfluidic platform. However, improvements are needed in the downstream processing steps. The cell- and insulin-loaded microparticles are expected to exhibit spherical shape, stability, high co-encapsulation efficiency (>80%), minimal loss of insulin stability, and optimal cell viability. Cell and insulin-loaded microparticle formulation should be further studied to improve and to support cell viability and functions, with the ultimate goal of assisting wound healing and alleviating the burden on healthcare systems.

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# P52. 3D-printed matrices for the purification of plasmid DNA

Silva, Joana (1); Sousa Rosa, Sara (1); Prazeres, Duarte M. F. (1); Silva-Santos, A. Rita (1);
Azevedo, Ana M. (1)

(1) iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal
E-mail: joanacsilva@tecnico.ulisboa.pt

Plasmid DNA (pDNA) has emerged as a valuable tool in various therapeutic applications, such as gene therapy and DNA vaccination. The production of high-quality plasmid DNA for these purposes requires an efficient and reliable manufacturing process. In this study, we aimed to investigate the potential of steric exclusion chromatography (SXC) for the capture of pDNA immediately after cell lysis, utilizing novel 3D-printed chromatographic matrices.

SXC is an alternative mode of chromatographic retention that operates on the principle of mutual steric exclusion, employing polyethylene glycol (PEG) as the exclusion agent. Unlike precipitation with PEG, SXC utilizes a hydrophilic solid phase as a nucleation center for biomolecules, promoting their accretion rather than forming precipitates. The aim of this study was to explore the influence of PEG molecular weight and concentration on the retention of pDNA using both conventional and 3D-printed matrices.

The experimental workflow involved cultivation of E. coli for pDNA production, followed by cell harvesting and lysis. PEG precipitation tests were conducted before investigating the potential of steric exclusion chromatography (SXC) for plasmid DNA (pDNA). By testing different molecular weights and concentrations of PEG in the precipitation process, it's possible to analyze the influence of these parameters on the yield and purity of the captured pDNA. This allows the identification of the optimal conditions for precipitation, leading to the maximum recovery of pDNA. Finally, the precipitation tests enabled us to make a comparative analysis, providing insights into the potential advantages of SXC over PEG precipitation. The performance of the 3D-printed matrices was compared to conventional matrices in terms of yield and purity of the captured pDNA.

The findings from this study provide valuable insights into the application of steric exclusion chromatography using 3D-printed matrices for the purification of plasmid DNA. The optimized conditions identified herein hold promise for enhancing the manufacturing process of various advanced therapies, including RNA vaccines, viral vectors, and cell and gene therapy research. The implementation of this technique can contribute to the production of high-quality grade plasmid DNA, meeting the stringent requirements of good manufacturing practice (GMP) and facilitating the development of novel therapeutic approaches.

# P53. Exploring The Role of Sacsin and S100B chaperones in Cytoskeleton Organization in ARSACS Disease

Boasinha, Ana Sofia (1); Murtinheira, Fernanda (1); Macedo, Luana (1); Rodrigues, Mário (1); Gomes, Cláudio (1); Herrera, Federico (1)

(1) BioISI – Instituto de Biosistemas e Ciências integrativas, Faculdade de Ciências da Universidade de Lisboa, 1749-016, Lisbon, Portugal
E-mail: fherrera@fc.ul.pt

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a recessive neurodegenerative disorder caused by mutations in the SACS gene, resulting in truncated or defective forms of the 520 kDa multidomain protein sacsin. The biological role of sacsin is barely known, although it displays chaperone activity and is related to mitochondrial behavior and function. While ARSACS studies have focused on neuronal cells, we have recently observed that sacsin is highly expressed in astroglia and developed a glial cell model of ARSACS to study their role in the disorder [1]. Sacsin knockout leads to an accumulation of the intermediate filaments in the juxtanuclear area and an upregulation of the S100B chaperone. S100B can play a protective role in neurodegenerative disorders by interfering with the formation of toxic protein aggregates [2]. To further understand its role in ARSACS, wild-type and sacsin knockout C6 cells were transfected with S100B fused with a fluorescent reporter. Sacs-/- cells exhibited S100B accumulation near the intermediate filament aggregates. Additionally, we are studying the effects of S100B knockdown on ARSACS cell phenotype. Withaferin A, an inhibitor of vimentin organization, induces juxtanuclear aggregation of glial intermediate filaments resembling Sacs-/- cell phenotype. However, S100B levels did not increase in response to Withaferin A. We are currently exploring the differences between the genetic and pharmacological models of ARSACS. Our results may provide relevant information for the future treatment of ARSACS but also advance our basic understanding of the function of sacsin and S100B proteins in cytoskeletal and mitochondrial organization.

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# P54. SPAX8-related mutations disrupt the subcellular distribution of NKX6-2 transcription factor

Peralta, Pedro (1,2); Murtinheira, Fernanda (1,2); Torres, Vukosava (2); Pinto, Francisco (2); Rodrigues, Mario (2); Herrera, Federico (1,2)

(1) Cell Structure and Dynamics Laboratory, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal
(2) BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal
E-mail: peperalta@ciencias.ulisboa.pt ; fherrera@fc.ul.pt

NKX6-2 is a transcriptional factor involved in the cell fate of neurons, oligodendrocytes, and pancreas. NKX6-2 also functions as an important factor in the formation and maintenance of myelin. Based on sequence homology with other members of the NKX family, the protein structure has some noteworthy domains: N-terminus Tinman domain (TN), NKX homeobox, and C-terminus transcriptional domain (TAD) [1, 2]. Loss-of-function mutations affecting the homeobox and TAD domains cause a rare neurodegenerative disease named Spastic Ataxia 8 (SPAX8) characterized by hypomyelinating leukodystrophy. Sixteen SPAX8-associated mutations have been identified to date: 5 nonsense, 2 frameshift and 9 missense mutations. We are developing new cellular models of the disease to characterize the behavior and function of the various SPAX8-related mutations. Using site-directed mutagenesis approaches, we produced constructs carrying 4 truncated NKX6-2 variants (K41*, E189*, Q197*, W203*) and 1 missense mutation (R200W) fused to the Venus fluorescent reporter. The expression and subcellular distribution of full-length NKX6-2, the 5 SPAX8-related mutations and a truncated mutant lacking the TN domain (aa 35-277) was characterized using Western blot and immunocytochemistry. Additionally, we analyzed NKX6-2 activity as a transcriptional repressor by means of a new reporter construct where constitutive luciferase expression was put under the control of the NKX6-2 response element. SPAX8-related mutations show a disrupted subcellular localization and/or form protein aggregates. The loss of transcriptional repressor activity in the SPAX8-related mutant K41* and the truncated variant without the TN domain suggests that the TN domain may play an important role in NKX6-2 transcriptional activity, as it was previously suggested to form a transcriptional repressing complex with the Gro/TLE protein family [1, 2]. Our results lay the groundwork to explain the molecular mechanisms behind SPAX8 and the design of therapeutic strategies for this rare disorder.

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# P55. Protein Structure and Stability in Hydrated Betaine-based Deep Eutectic Solvents

Gomes, Inês (1); Galamba, Nuno (1)

(1) BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences of the University of Lisbon, C8, Campo Grande, 1749-016 Lisbon, Portugal
E-mail: fc55700@alunos.fc.ul.pt; njgalamba@fc.ul.pt

Deep eutectic solvents (DESs) are a relatively novel class of green tailor-made solvents, generally comprised of a hydrogen-bond (HB) donor and an HB acceptor, and characterized by a significant depression of the melting point relative to those of the individual components [1].

DESs have attracted much attention due to some of their properties, including, biodegradability, affordability, tunability, and ease of preparation, thus, holding the promise to overcome some of the hindrances of room temperature ionic liquids. DESs have been exploited in multiple applications including biocatalysis and biomolecular (cryo)preservation and stabilization [2]. Recent reports have shown that enzymatic activity can be enhanced by using DESs as the reaction media [3]. A pivotal question associated with biological applications concerns the minimum amount of water required to allow biomolecules to maintain their structure and function.

Here, the effect of the water content and temperature in the structure of a prototypical globular protein, Ubiquitin, was probed through molecular dynamics simulations. Optimized force fields for the DES are reported along with a molecular analysis of the structure of the biomolecules in water and in the hydrated and dehydrated DESs.

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# P56. Towards a score-based metabolite identification algorithm for extreme resolution mass spectrometry

Luz, João (1); Traquete, Francisco (1); Ferreira, António (1); Sousa Silva, Marta (1); Cordeiro, Carlos (1)

(1) FT-ICR and Structural Mass Spectrometry Laboratory, BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa
E-mail: jmluz@fc.ul.pt

Metabolomics holds great promise towards a thorough understanding of biological systems. However, the complex and heterogenous nature of the metabolome poses significant analytical and interpretation challenges. Unlike proteins and nucleic acids, metabolites have extremely diverse chemical natures, making it difficult to develop single techniques for large-scale simultaneous identification and quantification in biological systems.

The traditional approach is to reduce sample complexity by performing chromatographic separation before Mass Spectrometry analysis, a time-consuming process that may lead to biological information loss. Furthermore, for metabolite identification, multiple tandem mass spectrometry (MS/MS) steps are often needed, leading to a further loss of time and biological verisimilitude.

Extreme resolution analytic techniques such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) offer a potential workaround by allowing the direct injection workflow to be used in complex biological samples [1]. Through this approach, thousands of metabolites can be detected simultaneously, with extremely high mass accuracy and the possibility of resolving isotopic fine structure, leading to unambiguous molecular formula attribution. Thus, the only missing link becomes the structural metabolite identification, distinguishing between molecular isomers, in an extreme resolution workflow without chromatographic separation and MS/MS.

We propose to solve this problem by taking inspiration from probabilistic scoring algorithms developed for proteomics, like Mowse and Mascot [2-3], which discriminate between multiple candidate proteins taking advantage of pre-existing knowledge, searching a list of masses against a comprehensive and specific database and assigning a score to each candidate.

Our scoring algorithm takes advantage of the fact that some metabolites have unique mass values in the context of an organism-specific database, and as such can be unambiguously identified based only on sufficiently accurate mass measurements. We then extrapolate from this small pool of reliably identified metabolites by increasing the scores of closely related compounds. To establish these relationships, we employ a variety of metrics previously used in metabolomics for post-identification functional enrichment and other purposes.

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# P57. Unraveling the metabolome and lipidome of SARS-CoV-2 infection by FT-ICR-MS

Louro, Mariana (1); Sousa Silva, Marta (1); Cordeiro, Carlos (1)

(1) FT-ICR and Structural Mass Spectrometry Laboratory, Biosystems and Integrative Sciences Institute (BioISI),
Faculdade de Ciências, Universidade de Lisboa, Portugal
E-mail: mplouro@fc.ul.pt

The outbreak of SARS-CoV-2 pandemic led to a global movement of vaccination. However, the number of infections keeps rising among vaccinated people, even if morbidity and mortality were drastically reduced. Since viral infections are known to cause profound changes at the global metabolome level, we aim to investigate the effects of SARS-CoV-2 infection in human populations that show a high degree of vaccination to uncover metabolomic signatures that relate to infectiveness, re-infection and disease progression, from mild to severe cases [1].

Plasma samples were analyzed by an untargeted metabolomics approach using a 7T FT-ICR SolariX XR mass spectrometer. Separation between disease severity is possible, however, differentiation between non vaccinated individuals and vaccinated individuals is less clear, albeit the presence of some outliers on the non-vaccinated group. However, fatty acids were found to be some of the molecules that best discriminate these two populations. Therefore, a more in-depth characterization on the plasma lipidome was performed using both the FT-ICR and GC-MS. Following a lipid-specific extraction, it was possible to identify 382 lipids in both negative and positive mode by FT-ICR. The main fatty acids were then quantified by GC-MS. Unsupervised statistical analysis was performed on this data, and a clearcut separation between disease severity. High-resolution mass spectrometry shows, once again, an elevated potential for diagnosis and even prediction of disease severity, also highlighting the role of lipid metabolism changes in response to infection.
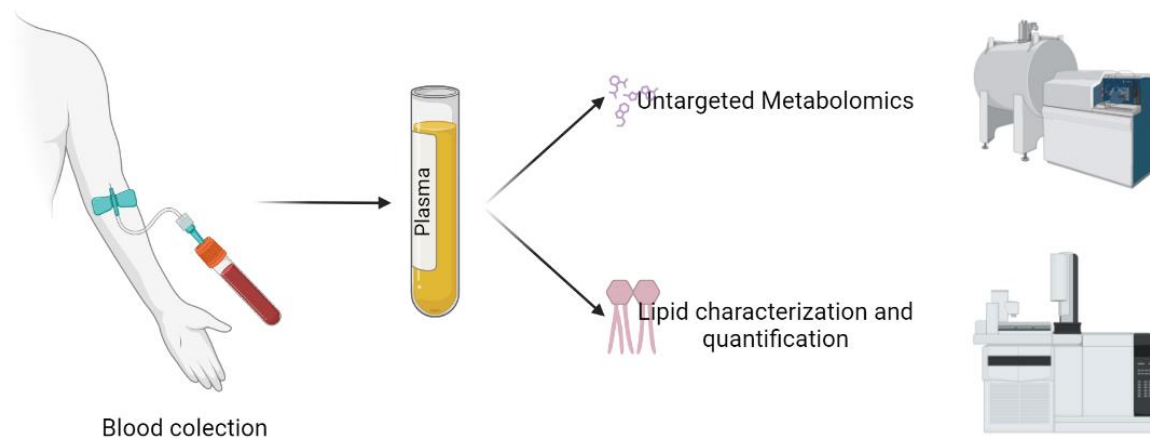


**Fig. 1.** Workflow and approaches used for plasma characterization of COVID-19 patients. Following blood collection, plasma was deproteinized and analyzed by FT-ICR using an untargeted approach. Lipids were extracted and samples re-analyzed by FT-ICR and GC-MS for identification and quantification of fatty acids.

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# P58. Unknotting the Mystery: Molecular Dynamics Simulations Unveil the Conformational Behaviour of UCH-L1

Ferreira, Sara G. F. (1); Faísca, Patrícia F. N. (2); Machuqueiro, Miguel (1)

(1) BioISI: Biosystems and Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) BioISI: Biosystems and Integrative Sciences Institute, Departamento de Física, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.
E-mail: sferreira@fc.ul.pt

The Ubiquitin C-terminal hydrolases (UCHs) are cysteine proteases involved in the hydrolysis of ubiquitin adducts in proteins, thereby counteracting the ubiquitination process. Among the UCH family members, UCH-L1 is particularly significant due to its high expression in the brain, accounting for approximately 1 to 5% of total neuronal protein [1], and also due to its association with neurodegenerative disorders such as Parkinson's and Alzheimer's, which has been attributed to its presence in Lewy bodies [2]. UCH-L1 is a single-domain protein consisting of 223 amino acid residues which possesses a complex 3D knotted structure, characterized by a '5 2' or 'Gordian' knot formed by five crossings of the polypeptide backbone. Initial findings suggest that UCH-L1 follows a dynamic folding pathway with unknotted intermediate states. During the unfolding process, the protein's α-helices unfold while the β-strands comprising the central hydrophobic core remain intact [3]. The role of the knot in maintaining this remarkable structural stability remains an open question. The present study aims to study the conformational space and mechanical stability of UCH-L1 using extensive molecular dynamics (MD) simulations. A computational protocol has also been developed to investigate several truncated versions of UCH-L1 with N- and C-terminus deletions, allowing assessment of the impact of the unknotting process on the protein's overall mechanical stability and its binding to ubiquitin. MD simulations were performed on both the wild-type UCH-L1 and truncated species in their apo and ubiquitin-complexed forms. The findings from this investigation will provide valuable insights into the structural and functional properties of UCH-L1, contributing to a better understanding of its involvement in neurodegenerative diseases.

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# P59. Inactivation methods of Bacillus spores for Mass Spectrometry

Gomes, Inês (1, 2); Silva, Marta (2); Cordeiro, Carlos (2)

(1) Instituto Universitário Militar, Centro de Investigação da Academia Militar, Unidade Militar Laboratorial de Defesa Biológica e Química, Lisbon, Portugal
(2) Laboratório de FTICR e Espectrometria de Massa Estrutural, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal
E-mail: fc59276@alunos.ciencias.ulisboa.pt

The response to bioterrorism events requires the use of fast and accurate detection methods to mitigate the risk and reduce the economic impact associated to these incidents. Recent developments in mass spectrometry (MS) technology allow not only an unequivocal detection and identification of microorganisms, with high sensitivity and specificity, but also a good characterization of biological agents with capacity to distinguish mutants within the same species, showing the discriminatory capacity of this technology [1] [2].

The genus Bacillus is a widely heterogeneous group characterized by rod-shaped bacteria, endospore-forming obligate or facultative aerobes. The genus Bacillus contains two important groups of bacteria: B. subtilis and B. cereus. B. cereus group include six distinct species: *B. anthracis*, *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, and *B. weihenstephanensis. B. cereus* is a food poisoning causative agent, and *B. anthracis* is a zoonotic agent with historical use in biological warfare and bioterrorism [3]. *B. anthracis* is considered a highly pathogenic bacteria (HPB) and the manipulation of this agent must occur in a biosafety level (BSL)-3 laboratory [4]. For safety reasons the analysis of HPB by MS requires complete inactivation of the samples, unless the mass spectrometer is operated in a biosafety level (BSL)-3 laboratory.

The scope of the present work is to test some well-established techniques for the inactivation of bacillus spores and to study the compatibility of the methods with mass spectrometry analysis. In this work, six pathogenic strains of *B. cereus* and 3 strains of *B. anthracis* were used. Several methods of spore inactivation and protein extraction were tested, namely: 1. formic acid (Bruker method); 2. formic acid with 0.22 µm filtration; 3. trifluoroacetic acid; 4. trifluoroacetic acid with 0.22 µm filtration; 5. Autoclaving. The samples in which complete inactivation was verified were later analyzed by Matrix-Assisted Laser Desorption Ionizatination - Time of Flight Mass Spectrometry (MALDI-TOF MS). The chosen method was the one that combined spore inactivation and the best information content of mass spectra.

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# P60. Examining the Influence of the S100B Chaperone on Mutant Huntingtin Aggregation

Botelho, Marta (1,2); Wanker, Erich E. (2); Herrera, Federico (1); Gomes, Cláudio M. (1)

(1) BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
(2) MDC – Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.
E-mail: fc53673@alunos.fc.ul.pt

Neurodegenerative diseases encompass a diverse group of disorders that progressively impair the structure and function of the nervous system. Huntington's disease (HD) is a fully penetrant neurodegenerative disorder caused by the dominantly inherited expansion of CAG trinucleotide repeats in the huntingtin gene. This genetic abnormality leads to the production and aggregation of mutant huntingtin protein (htt) containing a long polyglutamine stretch, which primes the disruption of crucial neuronal processes [1]. There are no current therapies to HD, mainly due to the lack of knowledge regarding the endogenous regulators of htt aggregation. The S100B protein is a calcium-binding alarmin predominantly synthesized by astrocytes, which novel chaperone activity inhibiting amyloid aggregation and mitigating toxicity was recently uncovered [2]. Consequently, S100B has emerged as a novel player in maintaining brain proteostasis in additional neurodegenerative diseases such HD.

To investigate this hypothesis, we firstly resorted to a FRET-based aggregation assay which revealed that S100B significantly delays and inhibits htt aggregation, while operating at suprastoichiometric concentrations. This led us to evaluate the effects of exogenous S100B on live cells expressing wild-type and mutant htt under aggregation conditions. HeLa cells transfected with wild-type/mutant htt show a correlation between the length of the polyglutamine stretch and the emergence of aggregation puncta/droplets in the cytoplasmic region. Cells expressing mutant htt will be treated with S100B to evaluate potential changes in the amounts and dynamics of htt inclusions. We expect to unravel the relationship between Huntington's Disease and S100B protein, which might constitute a foundation for the development of novel therapeutic approaches.

**References:** [1] McColgan, P. et al. (2017) Huntington's Disease: a clinical review. Eur. Neurol., 25, 24-34; [2] Cristóvão, J. et al (2019) S100 proteins in Alzheimer's Disease, Front. NeuroS., 13.

# P61. Development of biomanufacturing processes for non-viral protein nanocages with biotechnological and biomedical applications

João, Jorge (1,2); Sousa Rosa, Sara (1,2); Paulo, Pedro M. R. (3); Azevedo, Ana M. (1,2); Prazeres, Duarte Miguel F. (1,2)

(1) iBB – Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal
(2) Associate Laboratory i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal
(3) Centro de Química Estrutural, Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal
E-mail: jorgejoao@tecnico.ulisboa.pt

Non-viral protein nanocages (NVPNs) are highly ordered nanometer scale architectures, which are typically formed by homo- or hetero-self-assembly of multiple monomers into symmetric structures with different dimensions and morphologies [1]. Due to their very attractive intrinsic characteristics, several applications have been implemented. In particular, biotechnological and biomedical applications have been extensively investigated, showing that protein nanocages can be a promising and an interesting tool [2]. The ability to generate large amounts of pure and well-folded protein assemblies is crucial to transform nanocages into valuable bioproducts, whereby more efficient biomanufacturing processes are needed [3]. The main objective of this work was the development of scalable and cost-effective upstream and downstream processes for NVPNs (**Fig. 1**). Two models of NVPNs, one natural, the small heat shock protein from *Methanococcus jannaschi* (MjsHSP nanocages; 24 monomers, 12 nm and 396 kDa), and the other artificial, the trp RNA-binding attenuation protein (TRAP) nanocages (24 monomers, 22 nm and 2.2 MDa), were used. Regarding the production, both nanocages were produced in *Escherichia coli* and in an alternative expressing host (*Vibrio natriegens*). Several expression parameters were tested and optimized. In terms of the purification, chromatography was selected as the main strategy. Different approaches as well as chromatographic supports with distinct properties were evaluated. The NVPNs were analyzed by PAGE methods and characterized by dynamic light scattering, transmission electron microscopy, fluorescence correlation spectroscopy, and atomic force microscopy.
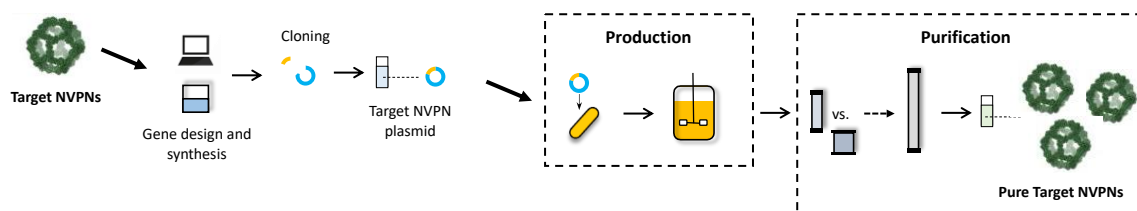


**Fig. 1.** Representation of a biomanufacturing process for NVPNs.

**References:** [1] Lee, E.J. et al. (2016) Bioengineered protein-based nanocage for drug delivery. Adv Drug Del Rev, 106, 157–171; [2] Diaz, D. at al. (2018) Bioengineering strategies for protein-based nanoparticles. Genes, 9, 370; [3] Theil, E.C. (2012) Ferritin protein nanocages-the story. Nanotechnol Percept, 8, 7–16.

# P62. Exploring Early Disease Mechanisms: Cross-Interactions between Aβ and Tau in Alzheimer's Disease

de Freitas, Daniela P. (1); Moreira, Guilherme G. (1,2); Figueira, António J. (1,2), Gomes, Cláudio M. (1,2)

(1) Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade Lisboa, 1749-016 Lisbon, Portugal.
(2) Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
E-mail: dpfreitas@ciencias.ulisboa.pt

Alzheimer's Disease (AD) is one of the major healthcare challenges of this century. Two of its prototypical hallmarks are a progressive neuroinflammation in the brain together with the appearance of proteinaceous aggregates. These are mostly formed by amyloid-β (Aβ) in extracellular plaques, and tau in intracellular neurofibrillary tangles. Despite their different localizations, tau exits cells and spreads throughout the brain in a manner that highly correlates with the evolution of brain damage during AD. This highlights the extracellular milieu as an environment where crucial molecular events might be happening, raising the possibility of interactions between extracellular misfolded tau and Aβ that could be relevant to understand AD development. Additionally, it is now known that protein dyshomeostasis starts from earlier, prodromal stages, and tau and Aβ pathologies interact synergistically. The lackluster success of current treatment strategies that mostly target late Aβ aggregates suggest that earlier aggregation events and players can be potentially relevant for novel approaches. In this sense, in the search for endogenous biological mechanisms that counteract Aβ and tau aggregation in the brain, S100B, a late-stage proinflammatory alarmin has emerged as an early-stage chaperone able to inhibit their *in vitro* aggregation and toxicity[1,2]. For this reason, we decided to explore cross-interactions between Aβ and tau by testing their *in vitro* co-aggregation mimicking early-disease scenarios. We made use of Aβ42 as a standard Aβ model, and of the Tau AD core (TADC), which comprises the core of tau fibrils and aggregates without the need of cofactors. This resulted in opposite effects: while Aβ aggregation was inhibited by tau, tau aggregation was accelerated by Aβ, displaying the complex interplay between Aβ and tau pathologies. On the other hand, while S100B inhibits both Aβ and tau *in vitro* aggregation individually, in a mixed context S100B action over tau is hindered, hinting to a competitive scenario between Aβ and tau as its clients, with aggregation curves having multiple growth phases suggesting multifaceted behaviors taking place. Overall, these results emphasize the complexity of the molecular events taking place in early AD, and highlight an opportunity for exploring them further, in order to build a more complete image of AD progression.

**References:** [1] Cristóvão, J. et al. (2019) The neuronal S100B protein is a calcium-tuned suppressor of amyloid-β aggregation. Sci Adv, 4, eaaq1702 [2] Moreira, G. et al. (2021). Nat Commun. 12; 6292.

# P63. Development of Inhalable mPEG-PLGA Nanoparticles for Antibody Loading as a Strategy for Lung Cancer Treatment

Viegas, Cláudia (1,2,3,4); Duarte, Sofia (3,4); Lourenço, J.P. (5,6); Grenha, Ana (2,6); Fonte, Pedro (2,3,4,6)

(1) Faculty of Medicine and Biomedical Sciences (FMCB), Universidade do Algarve, Gambelas Campus, 8005-139 Faro, Portugal
(2) Center for Marine Sciences (CCMar), Universidade do Algarve, Gambelas Campus, 8005-139 Faro, Portugal
(3) iBB—Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
(4) Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
(5) Centro de Química Estrutural (CQE), Instituto Superior Técnico, University of Lisbon, 1049-001 Lisboa, Portugal
(6) Department of Chemistry and Pharmacy, Faculty of Sciences and Technology, Universidade do Algarve, Gambelas Campus, 8005-139 Faro, Portugal
E-mail: viegas.claudiasofia@gmail.com; prfonte@ualg.pt

Lung cancer has a high mortality rate among all common cancers, estimated to be responsible for about 1 in 5 cancer deaths [1]. Conventional therapies are usually administered intravenously with low selectivity for tumour cells, which lead to potential side effects. Therapeutic antibodies are useful in treatment due to their higher specificity and bioactivity, and lower toxicity compared to small molecule drugs. Antibody encapsulation into nanoparticles for pulmonary delivery is a promising strategy, which combines targeted and controlled drug delivery with the ability to protect antibody structure and bioactivity [2]. Thus, the aim of this work was the development of mPEG-PLGA nanoparticles formulated into a dry powder by spray-drying aimed at localized lung cancer treatment. The optimization of nanoparticles followed a Design-of-Experiment (DoE) approach to target the desired features: small particle size and good colloidal stability. The polymer mass and surfactant concentration were considered as variables with a significant effect on nanoparticles properties, namely in particle size. The optimized nanoparticles were produced with 150 mg mPEG-PLGA and 1% Tween®80, presenting the lowest particle size of $\approx$ 300 nm, polydispersity index of $\approx$ 0.200, and zeta potential of $\approx$ -25 mV, considered suitable features for antibody encapsulation. The spray-drying optimization revealed that D-mannitol and L-leucine, used in combination, were the best matrix excipients to obtain microparticles. Their use at concentrations of 2% and 1% (w/v), respectively, allowed an increase in the yield up to $\approx$60% and reduction in powder adhesion to the apparatus walls due to leucine ability as dispersibility enhancer. Further studies will focus on antibody loading, aimed at establishing an inhalable lung cancer therapy [3]. So, spray-dried microencapsulated nanoparticles aimed at antibody delivery are herein reported for the first time.

**References:** [1] Siegel, R. et al. (2023) Cancer statistics. CA Cancer J. Clin., 73, 17-48; [2] Sousa, F. et al. (2017) A new paradigm for antiangiogenic therapy through controlled release of bevacizumab from PLGA nanoparticles. Sci. Rep., 7; [3] Marante, et al. (2020) An overview on spray-drying of protein-loaded polymeric nanoparticles for dry powder inhalation. Pharmaceutics, 12.

# P64. Probing the Anti-Sickling Power of Distinct Drugs in Sickle Cell Disease

Neto, Vasco M. S. (1); Galamba, Nuno (1); Victor, Bruno (1)

(1) Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade Lisboa, 1749-016 Lisbon, Portugal.
E-mail: fc52712@alunos.fc.ul.pt; blvictor@fc.ul.pt; njgalamba@fc.ul.pt

Sickle cell disease (SCD) is a debilitating blood disorder that affects millionsof people in sub-Saharan countries and other regions of the world. A missense mutation in the β-globin gene that codes for adult hemoglobin (HbA) results in a Glu-β6→Val-β6 substitution in the β-globins. This mutation prompts the aggregation of the deoxygenated form of this variant (deoxy-HbS), leading to hemolysis, painful vaso-occlusive crises, and regular infections. Despite recent developments in stem cell and gene therapy research, and the approval of anti-sickling drugs (i.e., Voxelotor), limitations persist. A potential therapeutic strategy involves the design of molecules that block protein-protein contacts, delaying the nucleation stage of deoxy-HbS. The most widely explored target for such a drug is the hydrophobic pocket formed by several amino acids in the β-globin where the Val-β6 of a neighbor molecule inserts itself, forming the lateral contacts in HbS fibers. Herein, we studied, through molecular dynamics, pocket-selectivity and the residence-time of several small-molecule drugs previously identified in experimental, in silico or in this study. Our results show that Alexidine, a recently reported antisickling molecule [1] displays high residence times and interacts strongly with both the residues of the hydrophobic pocket region of interest and the nearby heme group, potentially stabilizing the interaction. As such, it or other similar molecules may hold the key to blocking HbS aggregation and provide therapeutic advantages to this debilitating disease.
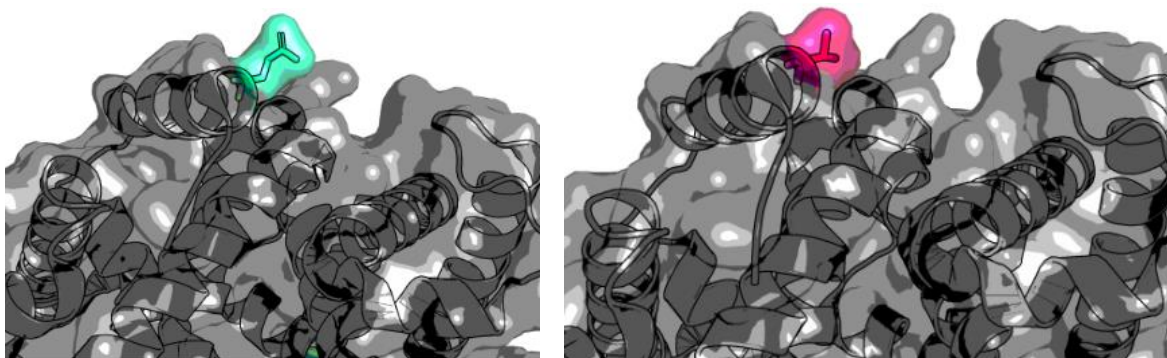


**Fig. 1 & 2.** (Left) Structure of Hemoglobin A with the βGlu6 residue highlighted; (Right) Structure of Hemoglobin S with the βVal6 residue highlighted.

**References:** [1] Metaferia, B. et al. (2022) Phenotypic screening of the ReFRAME drug repurposing library to discover new drugs for treating sickle cell disease. Proceedings of the National Academy of Sciences of the United States of America, 119, e2210779119.

# P65. Fingermarks: The new standard for clinical metabolomics?

Cruz, Marta (1); Louro, Mariana (1); Sousa Silva, Marta (1); Cordeiro, Carlos (1)

(1) FT-ICR and Structural Mass Spectrometry Laboratory, Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Portugal
E-mail: martacruz@alunos.fc.ul.pt

The human metabolome is a highly dynamic entity, reflecting very closely the phenotype, therefore allowing us to detect physiological and pathological alterations in real time. A fast, easy, cost-effective and non-invasive method to access the human metabolome is through fingermarks. Fingermarks are a complex mixture of endogenous compounds, such as lipids, vitamins, amino acids, and small proteins, produced by different types of glands, as well as semi-endogenous compounds like drugs [1]. Most literature reports on the use of fingermarks are in forensics, as fingermarks untargeted metabolomics can discriminate individuals by sex and age, and detect exogenous substances, such as drugs [2]. Recently, fingermark proteomics was used to diagnose breast cancer, further supporting the potentiality of the use of this biological sample in a clinical setting [3]. To test the potential of fingermark metabolomics as an alternative to plasma analysis, the golden standard for disease diagnosis and monitoring, we performed a comparative study between fingermarks samples and a standard human plasma reference material, using extreme resolution FT-ICR mass spectrometry and an untargeted metabolomics approach. Preliminary results show the potential of this novel approach.
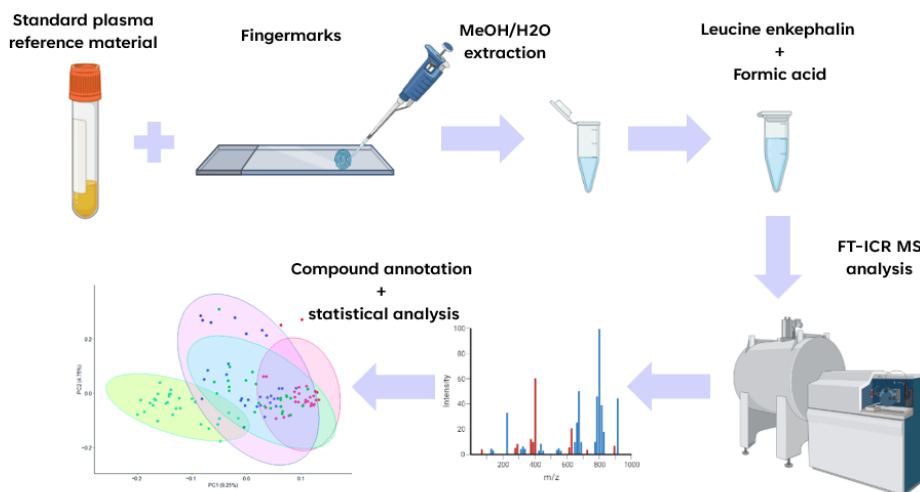


**Fig. 1.** Sample preparation, mass spectrometry and data analysis workflow.

**References:** [1] Assis, A. (2020). Caraterização química de impressões digitais humanas por FT-MS: Aplicações forenses; [2] Sousa Silva, M. et al. (2023) The invisible metabolic world of fingermarks revealed by Magnetic Resonance Mass Spectrometry (MRMS). Bruker Application Note, MRMS, 82; [3] Russo, C. et al. (2023) Non-invasive screening of breast cancer from fingertip smears-a proof of concept study. Sci Rep, 13, 1868.

# P66. The role of pH in the search for non-opioid analgesics: What we are doing about it and why

Sequeira, Joao G. N. (1,2); Roitberg, Adrian E. (2) Machuqueiro, Miguel (1)

(1) BioISI - Biosystems and Integrative Sciences Institute, Faculdade de Ciencias, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) Deparment of Chemistry, University of Florida, Gainesville 32603, USA
E-mail: jgsequeira@ciencias.ulisboa.pt

Acidosis is a fundamental characteristic of an inflammatory response [1] and should have an impact on the binding of compounds to all pH-sensitive targets. Acid-sensing ion channels (ASICs) are voltage-insensitive, proton-gated cation channels, widely expressed throughout the central and peripheral nervous system, that are involved in diverse physiological processes ranging from nociception to brain ischemia [2]. ASICs are activated by extracellular acidosis and ligands can act as antagonists or agonists for the channel's affinity for protons [3]. To discover ASIC activity modulators, it is crucial to understand the pH effects on the conformational rearrangement of the protein channel that leads to a change in the cation membrane permeability.

Constant-pH Molecular Dynamics (CpHMD) methods are pivotal to describe pH and its effects on the conformational space of biological systems [4]. The stochastic titration CpHMD (st-CpHMD) method has shown excellent performance over the years [4,5]. Until recently, our implementation of this method only supported the GROMOS 54A7 [4] and the CHARMM36m force fields [5]. We are currently working on the extension of this method to support the AMBER 14SB force field, an all-atom force field that is particularly suited for disordered proteins and nucleic acids. Here, we will discuss the intricacies of this state of the art method and our new extension, which aims to assist us in discovering non-opioid analgesics.

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# P67. Personalised Treatment for Cystic Fibrosis Patients with Rare CFTR Mutations

Coelho, Isabel (1); Rodrigues, Cláudia S (1); Pankonien, Ines (1); Amaral, Margarida D (1)

(1) BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, 1749-016 Lisboa, Portugal
E-mail: fc49395@alunos.fc.ul.pt; ipankonien@ciencias.ulisboa.pt ; msamaral@ciencias.ulisboa.pt

Cystic fibrosis (CF) is an autosomal recessive disease, and it is caused by variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes the CFTR protein, a chloride ($Cl^-$) and bicarbonate ($HCO_3^-$) anion channel expressed at the apical plasma membrane (PM) of epithelial cells [1-3]. The fluid and electrolyte homeostasis of many mucosal surfaces, including lung and intestine, is impaired in people with CF (pwCF), causing dehydration of epithelia and development of thick mucus. The life expectancy for pwCF is around 40 years and ongoing experience of health challenges throughout their lives, including chronic pulmonary infections, gastrointestinal disorders, and other complications, significantly impact their quality of life [4].

Until recently, therapies for CF focussed only on the symptoms but not on the underlying molecular defect caused by CFTR variants. With the recent development of small-molecule CFTR modulator drugs, a new era of CF therapeutics began. These modulators improve CFTR function by either correcting CFTR folding or potentiating the channel opening [5].

However, these drugs target the most common genotypes, leaving people with rare and uncharacterized variants with no possible treatment behind.

The aim of this project is to use the current CFTR modulators available namely Elexacaftor (VX-445, corrector), Tezacaftor (VX-661, corrector), and Ivacaftor (VX-770, potentiator), to find a possible treatment for the pwCF bearing orphan variants in a personalised manner. To accomplish this, patient-derived intestinal organoids from individuals carrying rare CFTR variants are generated from rectal biopsies and a Forskolin Induced Swelling (FIS) microscopy assay is performed in order to assess CFTR function for these individuals when treated with the current modulators.

To this end, organoids from an individual homozygous for p.Ala561Glu/p.Ala561Glu responded well to the modulators that were administered suggesting that this person can potentially benefit from the currently available therapies. As for organoids from an individual homozygous for p.Tyr1092X/p.Tyr1092X, there was no significant response to the modulators suggesting this individual would not benefit from the current therapies.

In conclusion, existing therapies can be repurposed for some pwCF with orphan variants. More organoids are currently being analysed to find personalised treatments for these pwCF so that their burdens associated with the disease can be relieved and their quality of life can greatly improve.

**References:** [1] De Boeck, K. et al. (2016) Progress in therapies for cystic fibrosis. Lancet Respir Med., 4, 662-674; [2] De Boeck, K. (2020) Cystic fibrosis in the year 2020: A disease with a new face. Acta Paediatr., 109, 893-899; [3] Cohen, T.S. et al. (2012) Cystic fibrosis: a mucosal immunodeficiency syndrome. Nat Med., 5;18, 509-519; [4] Elborn J.S. et al. (1992) JR. Cystic fibrosis: current survival and population estimates to the year 2000. Thorax., 46, 881-855; [5] van der Meer, R. et al (2021) CFTR Modulators: Does One Dose Fit All? J Pers Med., 24, 11, 458.

# P68. *In silico* study of promising ruthenium metallodrug delivery systems

Pires, Inês D. S. (1), Morais, Tânia S. (2), Machuqueiro, Miguel (1)

(1) BioISI - Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, Lisboa, Portugal.
(2) Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail: idpires@fc.ul.pt

Cancer has been rising to the top of the most prevalent and deadly diseases. Among breast cancer (BC) subtypes, the triple-negative (TN) is associated with high aggressiveness and poor prognosis[1]. Targeted therapies are already well established for the treatment of other BC subtypes, taking advantage of the expression of hormone receptors (estrogen and/or progesterone) and human epidermal growth factor receptor 2. As the TN subtype lacks their expression, its treatment is still heavily reliant on chemotherapy and cisplatin-like drugs. These are known for their lack of selectivity and tendency to promote the development of multidrug resistance.

TM34 is a ruthenium-based compound that has been suggested to be a more efficient and selective therapeutic compound than cisplatin [2]. TM34 derivatives have been in development the last years, in an attempt to increase their selectivity but preserve their activity, by adding a pH-sensitive linker and a peptide sequence that is recognized by receptor proteins from the FGFR family (overexpressed in TNBC cancers)[3]. Once in the presence of the altered pH of the tumor micro-environment, the linker hydrolyses and releases the active species.

This work aimed to study the impact of different substituent groups on the active specie's biophysical profile. This included examining the interaction of several TM34 derivative compounds with a membrane model (POPC) and calculating their membrane crossing energy profiles that can be used to estimate the membrane permeability coefficients. We used Molecular Dynamics simulations coupled with an Umbrella-sampling scheme to obtain the potential of mean force profiles, which allowed the calculation of the membrane permeability using the inhomogeneous solubility-diffusion model.

**References:** [1] Rakha, E.A. et al. (2009) Triple-negative/basal-like breast cancer: review, Pathology, 41(1), pp. 40–47. [2] Lin, K. et al. (2018) Applications of Ruthenium Complex in Tumor Diagnosis and Therapy, Frontiers in pharmacology, 9, 1323; [3] Machado, J.F. et al. (2020) Novel "ruthenium cyclopentadienyl"–-peptide conjugate complexes against human FGFR (+) breast cancer, Dalton transactions, 49, 5974–5987.

# P69. The Role of CFTR in Intestinal Epithelial Differentiation

Reis, João (1); Railean, Violeta (1); Pankonien, Ines (1); Amaral, Margarida D (1)

(1) University of Lisboa, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Lisboa, Portugal
E-mail: fc52723@alunos.fc.ul.pt ; ipankonien@ciencias.ulisboa.pt ; msamaral@ciencias.ulisboa.pt

Cystic fibrosis (CF) is the most common life-shortening monogenic disease in Caucasians. It is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which lead to defects in the corresponding protein, a $CL^-$ and $HCO_3^-$ channel [1]. p.Phe508del, the most common CF-causing variant (accounting for around 70% of all cases in Caucasian populations [2]), leads to an impaired traffic of CFTR. Besides its function as anion channel, CFTR has also been associated to other cellular processes, one of those being epithelial differentiation/polarization [3]. Recent data showed that dysfunctional CFTR impairs airway epithelial differentiation, in particular towards ciliated cells. However, it is still not known if the same happens in the intestine, another organ affected by CF [4].

The aim of the MSc work is to investigate the role of CFTR in intestinal epithelial differentiation and to determine if this process is affected when CFTR is dysfunctional.

Differentiated intestinal 2D-monolayers derived from 3D-organoids (generated from rectal biopsies) are being established. The differentiation of 2D-monolayers from both wildtype-CFTR and mutated-CFTR organoids is characterized using qPCR, Western Blot, and immunofluorescence, to assess the expression levels of CFTR, epithelial markers, and intestinal epithelium-specific cell type markers. Transepithelial electrical resistance measurements are also performed to assess the integrity and permeability of the 2D-monolayers. Furthermore, CFTR function of the 2D-monolayers, as well as their response to CFTR drugs, will be analyzed using the Ussing Chamber technique.

2D-monolayers derived from p.Phe508del-CFTR organoids were already successfully generated and differentiated. For analysis, cells were collected at different stages of differentiation: undifferentiated (day 0), partially differentiated (day 4) and fully differentiated (day 7). qPCR analysis was performed on expression levels of markers, such as TJP1 (tight junctions), CDH1 (adherens junctions), HES1 (colonocytes), KLF4 (goblet cells), SOX9 (deep crypt secretory cells), NEUROG3 (enteroendocrine cells) and LGR5 (crypt base columnar cells). A differential expression of these genes along the differentiation process has been observed, with TJP1 and SOX 9 levels increasing towards the end of differentiation, while CDH1 and HES1 levels decrease. KLF4 and NEUROG3 expression levels increased from the undifferentiated to the partial differentiation stage and then decreased on day 7, whereas for LGR5, the opposite happened (expression is lowest at partial differentiation). Further analyses are being performed to confirm the current results.

**References:** [1] Boyne, K. et al. (2022) Carbonic anhydrase and soluble adenylate cyclase regulation of cystic fibrosis cellular phenotypes. Am J Physiol Lung Cell Mol Physiol, 322, 333–347; [2] Cystic Fibrosis Genetic Analysis Consortium, "Cystic fibrosis mutation data base", http://www. genet. sickkids. on. ca/cftr/ (accessed in 7 June 2023) [3] Amaral, M. D. et al. (2020) What role does cftr play in development, differentiation, regeneration and cancer?. International Journal of Molecular Sciences, 21, 3133; [4] De Boeck, K. et al. (2016) Progress in therapies for cystic fibrosis. Lancet Respir Med, 4, 662–674.

# P70. Investigating the Role of Novel candidate HIV Viral miRNAs in Infection

Luta, Raquel (1); Ruivinho, Carolina (1); Andreia Jesus Amaral (2,3); Ana Espada de Sousa (4); Gama-Carvalho, Margarida (1)

(1) BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal
(3) Laboratório Associado para a Ciência Animal e Veterinária (AI4Animals), Avenida da Universidade Técnica, 1300-477 Lisboa,
(4) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal
E-mail: raluta@fc.ul.pt

MicroRNAs (miRNAs) are essential gene expression regulators that act by repressing translation and destabilizing mRNA molecules encoded by genes associated with various biological processes, including viral infections. Our lab performed an extensive characterization of small RNAs expressed in HIV-1 and HIV-2 infected human CD4 T cells [1], which led to the identification of three potential viral miRNAs, two encoded by HIV-1 and one by HIV-2. Using expression vectors encoding the predicted miRNA precursor sequence, we were able to show that two of these molecules exhibit characteristics of authentic miRNAs, including the requirement of hairpin formation for expression and Dicer-dependent processing [2]. Analysis of predicted miRNA targets identified potential regulatory events that could impact viral proteins, suggesting that these miRNAs may promote infection by inhibiting host proteins unfavorable to the virus. We are currently performing the functional characterization of these candidate miRNAs to test their ability to regulate host genes and influence HIV replication. For this purpose, we have generated seed sequence mutants that will be used to demonstrate the specific nature of observed effects. Impact of the expression of wild-type and mutant constructs on predicted host mRNA targets will be monitored by qRT-PCR and western blotting in HEK-293 and human T cell lines. The Jurkat T cell line, which harbors a latent HIV-1-GFP proviral genome will be further used to evaluate the impact of the candidate miRs in viral gene expression. Deepening our understanding of viral-host interactions and the regulatory mechanisms involved in HIV infections could lead to the development of innovative therapeutic strategies and provide insights into the dynamics of these viral infections, which is the main goal of this work.

**References:** [1] Amaral, A. J. et al. (2017) miRNA profiling of human naive CD4 T cells links miR-34c-5p to cell activation and HIV replication. EMBO J. 36, 346-360; [2] Ruivinho (2022) Functional characterization of putative miRNA-like sequences encoded in the HIV genome. MSc dissertation. hdl.handle.net/10451/55527.

# P71. Novel Combined Approaches to PTC Mutation Correction in CFTR

Henriques, Pedro (1); Clarke, Luka (2)

(1) Faculty of Sciences of the University of Lisbon (FCUL);
(2) Functional Genomics and Proteostasis (FunGP) lab, BioISI - Biosystems & Integrative Sciences Institute, FCUL (C8 Bldg)
E-mail: henriquespedro00@gmail.com ; laclarke@fc.ul.pt

Cystic Fibrosis (CF) is a life threatening disease that affects mostly caucasian populations. The disease, caused by deleterious mutations on the CFTR gene, is known to cause thickening of the mucus in the lung epithelia, causing cysts and frequent lung infections [1].

Of all CFTR variants, only 8,4% are nonsense mutations (class I) which are assossiated with premature termination codons (PTCs). These often produce severe phenotypes of CF, due to two major effects: the presence of the PTC which can lead to the creation of a truncated protein; and the dependent nonsense mediated decay (NMD) mechanism which targets and degradates PTC bearing CFTR transcripts [2].

In this work, a library of known compounds, comprizing G418 (geneticin); SMG1a; PTC124 (antaluren); RTC13; Amlexanox; and a novel compound library comprized of 52 compounds where used, in order to tackle PTC mutations in CFTR [3];[4]. The compounds were used against cells containing 3 PTC variants of CFTR: G542X; Y122X; and W1282X [5]. In a first phase, compounds were screened separately, to select the best ones, then, in a second phase, compounds will be screened in conjunction to observe if there is any ehanced impact in the abundance of mRNA and its structure (full length CFTR).

Screening was done by first using fluorescence microscopy on HEK cells containing a G542X mini-gene with a mCherry and GFP reporter in order to evaluate CFTR mRNA abundance and full length mRNA presence respectively.

Preliminary results show that G418 and SMG1a have the best efficacy out of the known compounds in both mRNA abundance and full length CFTR presence. Of the novel compounds, four seem to wield positive results being compounds 10, 11, 17 and 24.

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# List of Participants

| | |
|---|---|
| Abdullahi Adeyemi Muiz | aamuiz@fc.ul.pt |
| Adolfo Duarte Beles Viegas Amante Gonçalves | adolfoduartegoncalves@gmail.com |
| Alejandro Ornelas | fc60499@alunos.fc.ul.pt |
| Alexandra Sagatova | alexandra.sagatova@stuba.sk |
| Ana Beatriz dos Santos Pinção | fc58482@alunos.ciencias.ulisboa.pt |
| Ana Beatriz Martinho Lopes | abm.lopes@campus.fct.unl.pt |
| Ana Carolina Brasileiro Patrício | ana.patricio@tecnico.ulisboa.pt |
| Ana Carolina Coimbra Morais | carolina.morais08@gmail.com |
| Ana Catarina Ramalho | a.catarina.ramalho@insa.min-saude.pt |
| Ana Coutinho | ana.coutinho@tecnico.ulisboa.pt |
| Ana Cristina Belo Parente Henriques | fc53043@alunos.fc.ul.pt |
| Ana Isabel Carreiro Furtado | ai.furtado@campus.fct.unl.pt |
| Ana Loureiro | a.tloureiro2017@gmail.com |
| Ana Marta de Matos | amamatos@ciencias.ulisboa.pt |
| Ana Paula Carvalho | ana.carvalho@fc.ul.pt |
| Ana Rita da Silva Santos | ana.rita.santos@tecnico.ulisboa.pt |
| Ana Silveira Viana | anaviana@fc.ul.pt |
| Ana Sofia Boasinha Ribeiro | ana.sofia.ribeiro1@edu.ulisboa.pt |
| Ana Sofia Vieira dos Reis Ferreira de Oliveira | fc53036@alunos.fc.ul.pt |
| André Abrantes da Costa | aacosta_canedo@hotmail.com |
| André Miguel da Silva Fernandes | fc55342@alunos.fc.ul.pt |
| André Miguel Sousa Moleiro | fc54710@alunos.fc.ul.pt |
| Andreia Barreto | acbarreto@ciencias.ulisboa.pt |
| Andreia Bento de Oliveira | abdoliveira@fc.ul.pt |
| Andreia Cristina Janeiro | acjaneiro@fc.ul.pt |
| Andreia Jesus Lopes Fortuna | ajfortuna@fc.ul.pt |
| Andreia Valente | amvalente@fc.ul.pt |
| António Ferreira | aeferreira@ciencias.ulisboa.pt |
| António José de Jesus Matos Figueira | ajfigueira@fc.ul.pt |
| Arthur Slonina | arthur.slonina@etu.chimieparistech.psl.e |
| Bárbara de Sousa Marques | fc54680@alunos.fc.ul.pt |
| Bárbara Joana Henriques | bjhenriques@fc.ul.pt |
| Beatriz Domingues Farinha | beatriz.farinha@edu.ulisboa.pt |
| Beatriz Pereira Machado | bp.machado@campus.fct.unl.pt |
| Beatriz Pires Raso | fc56594@alunos.fc.ul.pt |
| Brenda Gabriella França | bgabriellaf@gmail.com |
| Bruna Santos | santosbrunaf@hotmail.com |
| Bruno Rocha Quintelas | fc56586@alunos.fc.ul.pt |
| Camilla Fonseca Silva | camillafonsecasilva@hotmail.com |
| Carina Isabel Bento Marques Coelho | cmcoelho@lip.pt |
| Carla D. Nunes | cmnunes@fc.ul.pt |
| Carlos Bernardes | cebernardes@ciencias.ulisboa.pt |
| Carlos M. Farinha | cmfarinha@fc.ul.pt |

| | |
|---|---|
| Carlos Manuel Barata da Fonseca Borges | fc13450@alunos.fc.ul.pt |
| Carolina Ferreira Fernandes | carolinaffernandes20@gmail.com |
| Chloé Galipeau | chloe.galipeau@edu.ulisboa.pt |
| Cláudia Alexandra da Silva Rodrigues | cadrodrigues@fc.ul.pt |
| Cláudia Sofia Mestre Viegas | viegas.claudiasofia@gmail.com |
| Cláudio M. Gomes | cmgomes@fc.ul.pt |
| Constança Pimenta | constancalourencopimenta@gmail.com |
| Daniel Filipe Fernandes de Carvalho | fc56597@alunos.fc.ul.pt |
| Daniel Valente-Matias | dfmatias@fc.ul.pt |
| Daniela Filipa Ribeiro Ferreira | ferreira-daniela@edu.ulisboa.pt |
| Daniela Neugebauer | dneugebauer@fc.ul.pt |
| Daniela P. de Freitas | dpfreitas@fc.ul.pt |
| Diana da Cunha Rodrigues Leitão | fc58467@alunos.fc.ul.pt |
| Diana Isabel Marques Vitorino | dianavitorino17@gmail.com |
| Diana Martins Carneiro | dianamcarneiro@alunos.fc.ul.pt |
| Diogo Alexandre Venâncio Cunha | diogocunha801@gmail.com |
| Diogo dos Santos Baptista | dsbaptista@fc.ul.pt |
| Diogo Manuel da Conceição Costa | dmc.costa@campus.fct.unl.pt |
| Domingos Morais | moraisbwill@gmail.com |
| Duarte Breia Clemente | duarteclemente@alunos.fc.ul.pt |
| Duarte Nuno Caçador Borralho | fc51349@alunos.fc.ul.pt |
| Eduardo Miguel Pereira Paes | fc56609@alunos.fc.ul.pt |
| Elif Dicle Çuhadar | elif_dicle@icloud.com |
| Elisabete Ribeiro Silva | ersilva@ciencias.ulisboa.pt |
| Federico Herrera | fherrera@fc.ul.pt |
| Fernanda Ivanira Henriques Murtinheira | fiferreira@fc.ul.pt |
| Filipa Moraes | filipatrigomoraes@gmail.com |
| Filipa Pedro Costa Santos | fpsantos@fc.ul.pt |
| Filipe Eduardo Pequito Rodrigues | filipe8.rodrigues@gmail.com |
| Filomena Martins | filomena.martins@ciencias.ulisboa.pt |
| Francisco Maria Reis Ventura Rosado Traquete | fmtraquete@fc.ul.pt |
| Francisco Pedro Esteves Duarte | fpduarte@ciencias.ulisboa.pt |
| Francisco Rodrigues Pinto | frpinto@fc.ul.pt |
| Gabriel Frederico Ferreira Martins | gfmartins@fc.ul.pt |
| Guilherme Moreira | ggmoreira@ciencias.ulisboa.pt |
| Hassan Ali | hali@utb.cz |
| Helena Monteirinho Leitão | helenaleitao1998@gmail.com |
| Henrique Miguel Correia Costa | fc49741@alunos.fc.ul.pt |
| Inês de Andrade Matos Gonçalves Saraiva | ines.saraiva@medicina.ulisboa.pt |
| Inês de Oliveira Feliciano | idfeliciano@fc.ul.pt |
| Inês Domingos Silva Pires | idpires@fc.ul.pt |
| Inês Filipe Gomes | inesfgomes31@gmail.com |
| Inês Lavado Gomes | fc59276@alunos.ciencias.ulisboa.pt |
| Ines Pankonien | ipankonien@ciencias.ulisboa.pt |
| Inês Sequeira Nunes Carolino | carolino.i135@gmail.com |
| Inês Vieira Peres Ventura | inesventura93@gmail.com |
| Íris Vaz de Lacerda | i.lacerda@campus.fct.unl.pt |
| Iryna Todoriko | iryna.riko@gmail.com |

| | |
|---|---|
| Isabel Maria Aveiro Coelho | fc49395@alunos.fc.ul.pt |
| Jaime A. S. Coelho | jaimeacoelho@edu.ulisboa.pt |
| Jéssica dos Santos Ribeiro de Freixo Cerqueira | jessica.cerqueira2000@gmail.com |
| Joana da Costa Silva | joanacsilva@tecnico.ulisboa.pt |
| Joana Justino Costa Martins | joanajcmartins@gmail.com |
| Joana Lourenço | fc51359@alunos.fc.ul.pt |
| Joana Silva Mendes | joana.silva.mendes@tecnico.ulisboa.pt |
| João André Isidoro Miranda | jamiranda@ciencias.ulisboa.pt |
| João Frederico S. Fernandes e Coelho Ferreira | jfsferreira@ciencias.ulisboa.pt |
| João G. N. Sequeira | jgsequeira@ciencias.ulisboa.pt |
| João Mendonça da Luz | jmluz@ciencias.ulisboa.pt |
| João Miguel Franco Machado | jmfmachado@ciencias.ulisboa.pt |
| João Nuno Marques Vitorino | jnvitorino@fc.ul.pt |
| João Pedro Lourenço Reis | fc52723@alunos.fc.ul.pt |
| João Pedro Prinas de Almeida Saavedra | joao.saavedra@tecnico.ulisboa.pt |
| Joaquim Manuel Trigo Marquês | jmtmarques@fc.ul.pt |
| Jorge Fernandes da Silva João | jorgejoao@tecnico.ulisboa.pt |
| Jorge Manuel Palma Correia | jmcorreia@fc.ul.pt |
| Katherine Bettencourt | fc56587@alunos.fc.ul.pt |
| Liana Silva | lianacsilva@ff.ulisboa.pt |
| Lucas Monteiro | fc52849@alunos.fc.ul.pt |
| Lúcia Santos | lasantos@ciencias.ulisboa.pt |
| Mafalda Alexandra Silva | mafalda.silva@insa.min-saude.pt |
| Mafalda da Fonseca Guimarães | fc54718@alunos.fc.ul.pt |
| Manuel Minas da Piedade | memp@fc.ul.pt |
| Manuela de Carvalho Kendall Pinto de Abreu | manuelakendall@outlook.com |
| Manuela Pereira | mmpereira@ciencias.ulisboa.pt |
| Marco Alexandre Pina de Sá | marcosa@alunos.ciencias.ulisboa.pt |
| Marcos Bento | mambento@ciencias.ulisboa |
| Margarida Alexandra Póvoa Lopes | margaridapovoalopes@gmail.com |
| Margarida Candeias Simões | margarida.csimoes@hotmail.com |
| Margarida Gama Carvalho | mhcarvalho@ciencias.ulisboa.pt |
| Margarida Sofia Costa Gomes | fc56637@alunos.fc.ul.pt |
| Maria Antónia de Oliveira Marcelo | mariamarcelo2000@gmail.com |
| Maria da Soledade C. S. Santos | mssantos@fc.ul.pt |
| Maria Estrela Borges Melo Jorge | mdjorge@fc.ul.pt |
| Maria Helena Garcia | mhgarcia@ciencias.ulisboa.pt |
| Maria Inês Romão | fc54357@alunos.fc.ul.pt |
| Mariana Ferreira Canhoto | mariana.fcanhoto@gmail.com |
| Mariana Foles Mendes | fc51347@alunos.fc.ul.pt |
| Mariana Isabel Francisco Alves | falves.mariana@gmail.com |
| Mariana Pintassilgo de Sousa Louro | mplouro@fc.ul.pt |
| Mariana Rodrigues dos Santos | fc52690@alunos.fc.ul.pt |
| Marisa Henriques Maria | marisahm1998@gmail.com |
| Marta Sofia de Oliveira Tavares de Freixo Gomes | marta.s.o.t.f.g@gmail.com |
| Marta Sofia Pedro Batista | martaspbat@gmail.com |
| Marta Sofia Pedroso da Cruz | martacruz@alunos.fc.ul.pt |
| Marta Sofia Silva da Cruz Botelho | fc53673@alunos.fc.ul.pt |

| | |
|---|---|
| Marta Sousa Silva | mfsilva@fc.ul.pt |
| Miguel Lobo Trigo | mtrigo100@gmail.com |
| Miguel Machuqueiro | machuque@ciencias.ulisboa.pt |
| Miguel Prudêncio | mprudencio@medicina.ulisboa.pt |
| Neuza Inês Guerreiro Salgado | neuzaines@hotmail.com |
| Nuno Galamba | ngalamba@gmail.com |
| Nuno Xavier | nmxavier@ciencias.ulisboa.pt |
| Olinda C. Monteiro | ocmonteiro@fc.ul.pt |
| Patrícia Alexandra Branco Fraga | p.fraga@edu.ulisboa.pt |
| Patrícia Pires | pmpires@ciencias.ulisboa.pt |
| Paulo J. Costa | pjcosta@ciencias.ulisboa.pt |
| Pedro Alexandre Rhodes Henriques | fc57627@alunos.fc.ul.pt |
| Pedro Almeida | palmeida@ciencias.ulisboa.pt |
| Pedro Emanuel Ferreira Peralta | peperalta@ciencias.ulisboa.pt |
| Pedro Filipe Gonçalves Rodrigues | fc48088@alunos.fc.ul.pt |
| Pedro Mateus | pmateus@itqb.unl.pt |
| Pedro Moreira | pedromoreira636@gmail.com |
| Rafaela Tenera Marques | rafaela.m.1996@gmail.com |
| Raquel Alexandra Gaspar Luta | raquelluta@gmail.com |
| Raquel Gonçalves Torres | raquel.torres.2001@gmail.com |
| Ricardo Henriques | |
| Ricardo Jorge Gonçalves Teixeira | rjteixeira@fc.ul.pt |
| Rita Alexandra Padinha Lopes | rita.padinha.lopes@tecnico.ulisboa.pt |
| Rita Dantas Rocha | rochadrita@gmail.com |
| Rita Tomé | aritome@ciencias.ulisboa.pt |
| Rui Daniel Freire Alonso | fc54695@alunos.fc.ul.pt |
| Ruy Manuel Soares de Albergaria Domingos | ruymdomingos@gmail.com |
| Sara Botelho Gutierrez de Lima | sb.lima@campus.fct.unl.pt |
| Sara Gabriela Ferraz Ferreira | sferreira@fc.ul.pt |
| Sofia de Oliveira Dias Duarte | sofia.duarte@tecnico.ulisboa.pt |
| Sofia Rocha Anão | sofia.ranao@gmail.com |
| Susana Santos | smsantos@ciencias.ulisboa.pt |
| Tânia Marques | trmarques@fc.ul.pt |
| Tânia S. Morais | tsmorais@ciencias.ulisboa.pt |
| Teresa Sofia Araújo Esteves | teresa.esteves@tecnico.ulisboa.pt |
| Tiago Pereira Gomes | tpereiragomes@hotmail.com |
| Tomás Ramalho Ginestal Monteiro | fc56606@alunos.fc.ul.pt |
| Ulácia | ulaciaduarte@icloud.com |
| Vanessa Morgado | vmmorgado@fc.ul.pt |
| Vanessa Xinyi Ji | vanessa.x.ji@tecnico.ulisboa.pt |
| Vasco Miguel dos Santos Neto | vasconeto25@hotmail.com |
| Vicente Vieira | vieira.fonseca.vicente@gmail.com |
| Violeta Railean | vrailean@fc.ul.pt |

# SPONSORS