Book of Abstracts

2nd Chem & Biochem Students Meeting

2^{nd} Chem & Biochem Students Meeting

Book of Abstracts

July 15^{th}, 2022

Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal

Title

Book of abstracts of the 2^{nd} Chem & Biochem Students Meeting

Editorial board

Andreia Fortuna, Inês Feliciano, João Machado, Tomás Silva, Vanessa Morgado

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Welcome Message

The Organizing Committee is pleased to welcome you to the **2nd 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.

This event aims at stimulating the scientific creativity and the exchange of ideas among the students from DQB and from related institutions in Lisbon area, as well as encourage the companionship and multidisciplinary work through the establishment of new research collaborations.

The program consists of oral, flash, and poster communications by PhD, MSc, and BSc students in the Chemistry and Biochemistry fields, who will share and discuss their research work with peers and with experienced researchers/professors. Prizes will be awarded to the best communications, selected by a scientific jury composed of three professors from DQB. In addition to the regular communications, the program also includes two invited plenary lectures from well-known researchers working on the frontiers of chemistry and biochemistry, as well as a Round Table discussion about Communication of Science, focusing on the challenges for the next generation of scientists.

The Organizing Committee wishes all participants a very fruitful meeting!

Lisbon, July 15th 2022,
The Organizing Committee

Registration: C8 Atrium

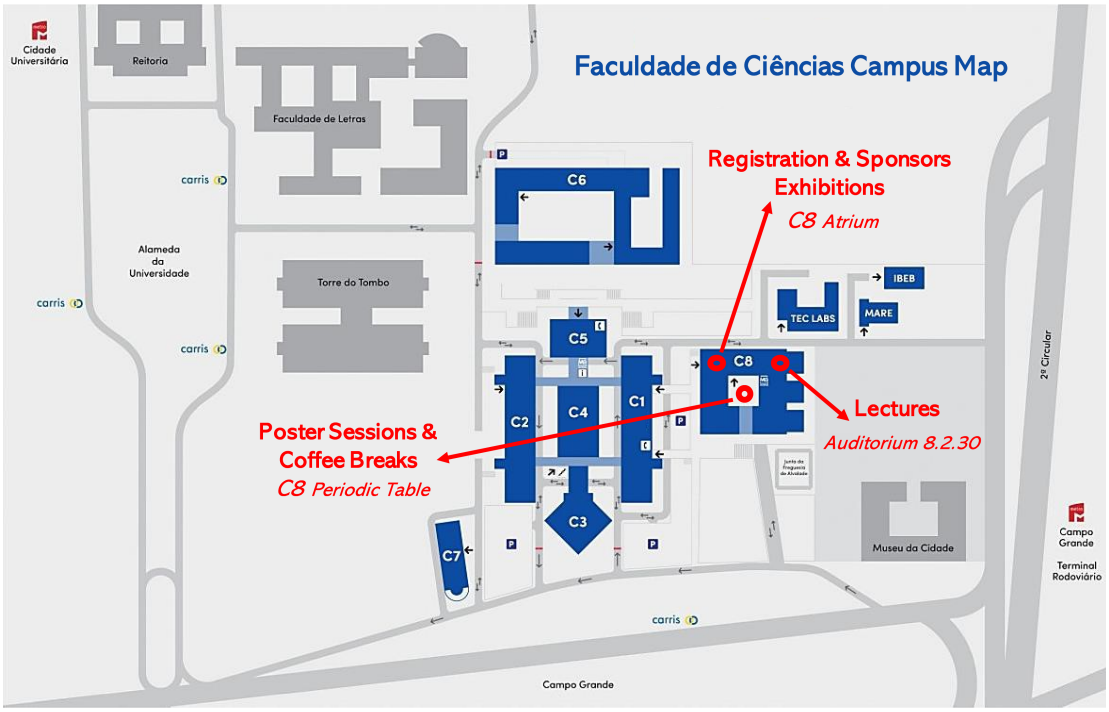
Sponsors Exhibitions: C8 Atrium

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



Faculdade de Ciências da Universidade de Lisboa Campo Grande, 1749-016 Lisboa, Portugal

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.

2nd Chem&Biochem Students Meeting

15th July 2022 | Faculty of Sciences, University of Lisbon (C8)

Final Program

Morning session

8:00 – 9:00 | Event Registration

9:00 – 9:15 | Formal Opening

Manuel Minas da Piedade | FCUL Chemistry and Biochemistry Department president

António Jorge Parola | SPQ – Portuguese Society of Chemistry

9:15 – 10:15 | Plenary Session I

PL1. Pedro Góis | Faculty of Pharmacy (ULisbon), iMed.Ulisboa - *New Chemistries for Stimuli-Responsive Targeting Drug Conjugates*

10:15 – 11:00 | Coffee break + Poster Session

11:00 – 12:00 | Oral Session I — Chair: Andreia Valente (FCUL)

O1. Sónia Santos - *The O-demethylation of 4-alkylguaiacols by Cytochrome P450 AgcAEP4*
O2. Jorge João - *Purification of small heat shock protein nanocages: development of a chromatographic strategy*
O3. Miriam Colaço - *Kinetic and thermodynamic studies of synthetic receptor-ligand complexes in water*
O4. Nuno Oliveira - *Novel US-CpHMD Protocol to Study the Protonation-Dependent Mechanism of the ATP/ADP Carrier*

12:00 – 13:00 | Flash Pitches — Chair: António Figueira (FCUL)

F1. Ricardo M. Silva - *Development and simulation of a purification platform based to deliver clinical-grade mesenchymal stem/stromal cell-derived extracellular vesicles*
F2. Ana R. Reis - *Electrochemical growth of Fe-MOF-74-type films*
F3. Susana Parreiras - *Design, production and characterization of antiviral proteins targeting SARS-CoV-2*
F4. Claúdio C. Fernandes - *Use of Hansen Solubility Parameters (HSPs) to investigate the solubility behavior of Hydrophilic and Hydrophobic NADES*
F5. Marta Batista - *Studying glycerol permeability through aquaporin from Plasmodium falciparum for the development of new antimalarial therapies*
F6. Catarina Nascimento - *On the Role of Polyphenols in the Hydration and Aggregation of Parkinson's Disease Related Peptides*
F7. Beatriz Lopes - *Understanding the molecular basis for human mitochondrial glutamyl-tRNA synthetase (hERAS2) deficiency – from recombinant protein production to structural characterization*
F8. Duarte B. Clemente - *Preparation of oxygenated metabolites of agrochemical active ingredients*
F9. Alexandre Coelho - *Development of a machine learning- based pipeline able to predict genes associated with diseases and cell processes using interpretable network embeddings*
F10. Luís C. Almeida - *Tailoring polydopamine and polynorepinephrine coatings for bacterial laccase based phenolic biosensor*

13:00 – 14:15 | Lunch break

Afternoon session

14:15 – 15:15 | Plenary Session II

PL2. Inês Figueira | Medical School (UNovaLisboa) - *Roadmap from blood to brain: low molecular weight phenolic metabolites as promising compounds against neurodegeneration*

15:15 – 16:15 | Oral Session II — Chair: Nuno Neng (FCUL)

O5. Sara Rosa - *A Machine Learning approach to optimise mRNA vaccines production*
O6. Ana C. Rocha - *Improving the reliability of oil spill identification by the accurate simulation of diagnostic ratios*
O7. Bruno Calçada - *Towards a framework to unify in silico methods for endocrine disruptors identification: a machine learning model to predict thyroid peroxidase inhibition*
O8. Adhan Pilon - *Iron-cydopentadienyl compounds with phosphane and N-based ligands show strong activity against a broad panel of human cancer cell lines*

16:15 – 17:00 | Coffee break + Poster Session

17:00 – 18:15 | Round Table — Moderator: Marta Santos (FCUL)

Communication of Science : The challenges of the new generations of scientists

Adriano Cerqueira | Antena 1 / FCSH-UNL
Joana Lobo Antunes | FCSH-UNL / IST-UL
Vera Novais | Jornal Observador

18:15 – 18:30 | Closing Session & Awards

Coordinations of FCUL PhD in Chemistry and Biochemistry

Plenary Lectures

PL1 | New Chemistries for Stimuli-Responsive Targeting Drug Conjugates

Pedro M.P. Gois

Research Institute for Medicines (iMed.ULisboa) Pharmacy Faculty, Universidade de Lisboa
Email: pedrogois@ff.ulisboa.pt

Targeting drug conjugates, emerged as a powerful class of chemotherapeutic agents that are capable of sparing healthy tissues by liberating the cytotoxic payload upon specific antigen recognition. A considerable body of work in this field highlighted that targeting drug conjugates therapeutic efficacy, correlates well with the conjugate homogeneity and activation of the drug at the diseased site. Therefore, the linker technology used to connect both functions contributes decisively to the therapeutic usefulness of these constructs. In this communication will be presented our most recent finding on the design of functional likers for targeting drug conjugates, based on boron complexes (B-complexes)[1] that can be modulated to exhibit fluorescence and to respond to glutathione, pH or reactive oxygen species stimulus. [2-4]

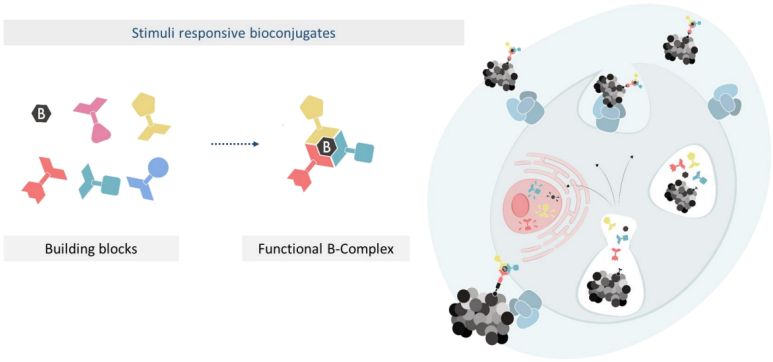


Figure 1: Modular, stimuli-responsive Targeting Drug Conjugates.

[1] J. P. M. António, Roberto Russo, C. P. Carvalho, Pedro M. S. D. Cal, Pedro M. P. Gois, Chem. Soc. Rev. 2019, 48, 3513.

[2] R. M. R. M. Lopes, A. E. Ventura, L. C. Silva, H. Faustino, P. M. P. Gois, Chem. Eur. J. 2018, 24, 12495.

[3] F. M. F. Santos, A. I. Matos, A. E. Ventura, J. Gonçalves, L. F. Veiros, H. F. Florindo, P. M. P. Gois, Angew. Chem. Int. Ed. 2017, 56, 9346.

[4] António, J. P. M.; Carvalho, J. I.; André, A. S.; Dias, J. N. R.; Aguiar, S. I.; Faustino, H.; Lopes, R. M. R. M.; Veiros, L. F.; Bernardes, G. J. L.; Silva, F. A.; Gois, P. M. P. Angew. Chemie Int. Ed. 2021, 60, 25914.

Acknowledgements: We thank FCT and FEDER LISBOA-01-0145-FEDER-029967 e PTDC/QUI-QOR/29967/2017

PL2 | Roadmap from blood to brain: low molecular weight phenolic metabolites as promising compounds against neurodegeneration

Inês Figueira (1) and Cláudia N Santos(1,2,3)

(1) NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal.
(2) iBET, Instituto de Biologia Experimental e Tecnológica, Av. da República, Apartado 12, 2781-901 Oeiras, Portugal,
(3) ITQB, Universidade NOVA de Lisboa, Oeiras, Portugal.
Email: ines.figueira@nms.unl.pt

Epidemiological and clinical studies over the past decades have highlighted the health benefits of diets rich in fruits and vegetables. These food sources are rich in (poly)phenols, natural compounds described to hold the potential to prevent and/or retard the development of chronic disorders as neurodegenerative ones. Indeed, neurodegenerative disorders comprise complex and multifactorial pathologies which are increasing and remain cureless. The possibility of altering the progression or the development of these diseases through diet is an emerging and attractive approach.

We believe that the prevention and treatment of neurodegeneration, characterized by a mechanistic complexity, will require novel multi-targeted therapeutic strategies, tackling different disease hallmarks. In that sense, dietary (poly)phenols can emerge as a reliable pleiotropic alternative. However, the precise contribution of dietary (poly)phenols and their metabolites to this modulation is still in its infancy. Absorption, blood concentrations, and metabolic fate of some (poly)phenols are quite uncertain, which can hamper the research in terms of understanding their effects. In fact, it is necessary to identify the bioavailable metabolites resulting from dietary (poly)phenols, as well as their ability to overcome the blood-brain barrier to reach the brain. The brain permeability of (poly)phenol metabolites abundant in circulation, as well as their molecular mechanisms on neurodegeneration and neuroinflammation, will be discussed.

This work was supported by "iNOVA4Health – UIDB/04462/2020 and UIDP/04462/2020, and by the Associated Laboratory LS4FUTURE (LA/P/0087/2020), two programs financially supported by Fundação para a Ciência e Tecnologia (FCT) / Ministério da Ciência, Tecnologia e Ensino Superior. We also acknowledge to European Research Council (ERC Starting Grant – LIMBo – 804229).

Oral Communications

O1 | The *O*-demethylation of 4-alkylguaiacols by Cytochrome P450 AgcAEP4

Santos, Sónia F (1,3); Bommareddy, Rajesh (1,3); Black, Gary W (1,3); Huang, Meilan (2); Singh, Warispreet (1,3)

(1) Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom
(2) Department of Chemistry & Chemical Engineering, Queen's University, Belfast, BT9 5AG, United Kingdom
(3) Hub for Biotechnology in Build Environment, Newcastle upon Tyne, United Kingdom

P450 enzymes are a superfamily of heme-containing monooxygenases. These enzymes have high catalytic versatility including the ability to catalyze the oxidation of organic substrates. [1] AgcA encoding a P450 CYP255A1 family upregulates the growth of bacterial strain Rhodococcus rhodochrous EP4 on 4-alkylphenol and is responsible for the meta-cleavage of the growth substrate. Experimental research has shown that the AgcAEP4 enzyme has a preference for bigger substrates such as 4-propylguaiacol over small substrates like guaiacol.[2] This enzyme was shown to have the opposite and complementary specificity of its homologous enzyme GcoA. 4-alkylguaiacol are lignin-derived products obtained by reductive catalytic fractionation (RCF) of corn biomass. Understanding this specificity in the catalytic reaction will be an asset in the design of enzymes for the lignin breakdown to extract valuable products as fine chemicals. To study the specificity in the catalytic reaction of AgcAEP4, we built a homology model using the GcoA enzyme, and then studied the preferred binding of analogous substrates 4-propylguaiacol, 4-methylguaiacol, 4-ethylguaiacol, and guaiacol, by the molecular docking, comprehensive MD simulations and QM/MM calculations.

Our study shows that AgcAEP4 adopts an open conformation, and therefore the enzyme has a bigger pocket to accommodate bigger substrates, allowing a water flux into the active site. MD simulations disclosed smaller substrates could not stay in the catalytic site, making the reaction unlikely to happen. Finally, our QM/MM study shows that the relative energy barrier associated with the hydrogen transfer is lowest for AgcAEP4 in complex with 4-propylguaiacol, compared to the enzyme in complex with the other smaller substrates.

[1] Dinis, P et al. (2019) Biomass, Biofuels, Biochemicals: Advances in Enzyme Technology, 389-418

[2] Fetherolf, M M et al. (2020), Proceedings of the National Academy of Sciences of the United States of America, 117, 25771-25778

The authors acknowledge the financial suppor from studentship project reference number ET20/HLS/APP/SINGH. We are grateful for the computing resources from QUB high performance computing Tier2 computing resource funded by EPSRC (EP/T022175)

O2 | Purification of small heat shock protein nanocages: development of a chromatographic strategy

João, Jorge (1,2); Sousa Rosa, Sara (1,2); 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, Lisbon, Portugal;
(2) Associate Laboratory i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Protein nanocages are versatile vehicles for drug delivery. These nanometer scale architectures feature an inner cavity that can be loaded with different drugs. The clinical development of protein nanocages requires large amounts of pure, well-folded assemblies [1]. However, while the currently used purification approaches are suitable for proof-of-concept studies at lab scale [2], biomanufacturing at large scale will require more efficient bioprocess technologies to enable the use of protein nanocages in clinical applications [3].

The main objective of this work was to develop scalable and cost-effective processes for the biomanufacturing of protein nanocages with particular emphasis in the downstream processing steps. The 16.5 kDa small heat shock protein from *Methanococcus jannaschii* (MjsHSP) was used as model. The *in vivo* assembly of 24 units of MjsHSP originates 12 nm nanocages with octahedral symmetry and demarcated exterior and interior surfaces. The nanocages were produced in E. coli and released by sonication. The purification strategy consisted of an intermediate purification followed by a polishing step to achieve a highly purified and formulated product. Different approaches of chromatography (anion exchange, size exclusion and multimodal) as well as traditional and novel chromatographic supports with distinct properties were tested and analysed. The pure MjsHSP nanocages were analysed by SDS-PAGE and characterized by dynamic light scattering, fluorescence correlation spectroscopy, transmission electron microscopy and atomic force microscopy. The obtained results demonstrated that a downstream processing strategy based on two chromatography steps could be an efficient platform to obtain pure protein nanocages for pre-clinical/clinical applications.

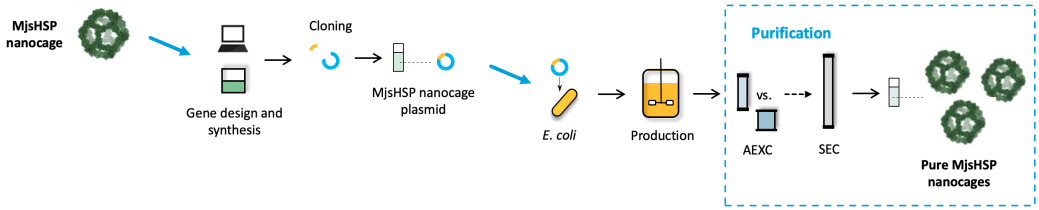


Figure 1: Graphical Abstract

[1] Theil, EC (2013) Nanotechnol Percept, 8, 7-16.
[2] Choi, S-H et al. (2011) Biomacromol, 12, 3099-3106.
[3] Lee, EJ et al. (2016) Adv Drug Del Rev, 106, 157-171.

Funding received for the iBB from FCT (Project UIDB/04565/2020 and UIDP/04565/2020) and from Programa Operacional Regional de Lisboa 2020 (Project N. 007317) and for the Associate Laboratory i4HB (Project LA/P/0140/2020). Funding from FCT through the PhD fellowships to Jorge João (PD/BD/150335/2019, BIOTECnico PhD Program) and to Sara Sousa Rosa (SFRH/BD/148437/2019).

O3 | Kinetic and thermodynamic studies of synthetic receptor-ligand complexes in water

Colaço, Miriam (1); Máximo, Patrícia (1); Parola, A. Jorge (1); Basílio, Nuno (1)

(1) Laboratório Associado para a Química Verde (LAQV), Rede de Química e Tecnologia (REQUIMTE), Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

Host-guest systems based on synthetic macrocyclic receptors (hosts) and small organic molecules or ions (ligands/guests) provide important supramolecular tools for the construction of self-assembled materials with diverse applications such as sensors, nanotechnological devices and molecular machines. Moreover, due to their relatively small molecular weight and well-defined structure, these systems provide relevant biomimetic models to investigate molecular recognition phenomena with a level of detail that can not be achieved in biomolecular systems.

In this communication, we present kinetic and thermodynamic studies on the formation of high-affinity host-guest pairs in water, based on cucurbit[8]uril (CB8) host and photoswitchable dithienylethene (DTE) guests [1,2]. We show that the 1:1 complexes with the colored ring-closed isomers are thermodynamically and kinetically more stable (ca. 100-fold) than the ones formed with the fluorescent open forms. We also show that the introduction of two negatively charged side arms in the DTE, that act as electrostatic kinetic barrier for the inclusion of the guest, slows down the kinetic constants by at least 5 orders of magnitude [3]. Following this initial study, we developed a system with an even stronger barrier towards the formation of rotaxanes and a method to easily synthetize them in situ. Additionally, these systems allow the observation and characterization of out-of-equilibrium complexes in unusual three-dimensional arrangements.

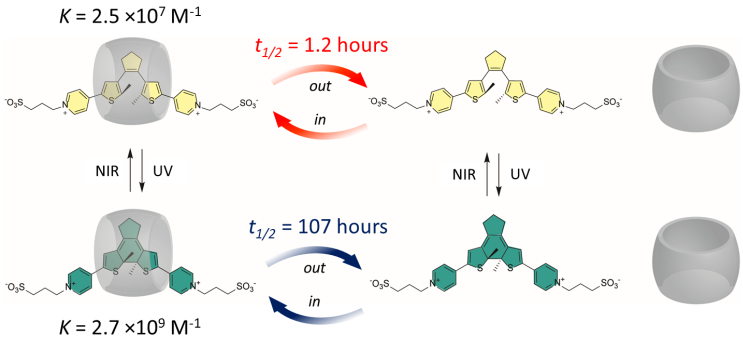


Figure 1: Light control over the displacement kinetics of the 1:1 complexes that CB8 forms with the DTE guest.

[1] Ferreira, P. et al. (2019) Chem.Eur.J., 25, 3477-3482;
[2] Máximo, P. et al. (2022) Org. Chem. Front., DOI: 10.1039/d2qo00423b;
[3] Colaço, M. et al. (2021) Chem. Eur. J., 27, 9550-9555.

This work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). FCT/MCTES is also acknowledged for supporting the National Portuguese NMR Network (ROTEIRO/0031/2013-PINFRA/22161/2016, co-financed by FEDER through COMPETE 2020, POCI, PORL, and FCT through PIDDAC) and for the grants PTDC/QUI-COL/32351/2017, PTDC/QUI-QFI/30951/2017 and CEECIND/00466/2017. The authors also acknowledge the PhD grant 2021.07205.BD and "Verão com Ciência" initiative from FCT – Foundation for Science and Technology and DGES – Direção Geral do Ensino Superior.

O4 | Novel US-CpHMD Protocol to Study the Protonation-Dependent Mechanism of the ATP/ADP Carrier

Oliveira,Nuno F. B. (1) and Machuqueiro, Miguel(1)

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

Electrostatic interactions are key participants in biomolecular processes, being the main driving force of molecular interactions. We developed a new protocol combining constant-pH molecular dynamics (CpHMD) simulations with an umbrella sampling (US) scheme (US-CpHMD) to study the mechanism of ADP/ATP transport (import and export) by their inner mitochondrial membrane carrier protein [ADP/ATP carrier (AAC)] [1]. This method helps overcome the time-scale limitations of regular MD simulations, while allowing the sampling of the most relevant protonation states in all molecules. Several computational studies have already identified the importance of electrostatics in this protein system [2], however, none have tackled the complete transport process pathway and all lack the correct description of pH.

The import of anionic substrates along the mitochondrial membrane is unfavourable due to a lower substrate concentration and an opposite membrane potential. These limitations may have created an evolutionary pressure on AAC to develop specific features benefiting the import of ADP. In our work, the potential of mean force profiles show that the substrate transport is energetically favourable, however there is a clear selectivity in the import of ADP compared to ATP [1]. Furthermore, the transient protonation of both substrates while going through the AAC cavity only occurs in the import process, which is an important feature to counteract the opposite mitochondrial membrane potential. Finally, we observed a substrate-induced disruption of the matrix salt-bridge network, which can promote the conformational transition required to complete the import process. This work unravelled several important structural features where the complex electrostatic interactions were pivotal to interpreting the protein function and illustrate the potential of applying the US-CpHMD protocol to other transport processes involving membrane proteins.

[1] Oliveira, NFB, et al. (2022) J Chem Inf Model., 62: 2550–2560.

[2] Bidon-Chanal, A, et al. (2013) J Phys Chem Lett., 4: 3787–3791.

We also acknowledge the financial support from the Fundação para a Ciencia e a Tecnologia through grants CEECIND/02300/2017 and 2021.06409.BD, and projects PTDC/BIA-BFS/28419/2017, PTDC/FIS-OUT/28210/2017, UIDB/04046/2020, and UIDP/04046/2020.

O5 | A Machine Learning approach to optimise mRNA vaccines production

Rosa, Sara Sousa (1,2); Nunes, Davide(3); Antunes, Luis(3); Prazeres,Duarte M.F. (1,2); Marques, Marco P.C.(4); Azevedo, Ana M. (1,2)

(1) Department of Bioengineering, iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
(2) Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
(3) LASIGE, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749 - 016 Lisboa, Portugal
(4) Department of Biochemical Engineering, University College London, Bernard Katz Building, Gordon Street, London WC1H 0AH, United Kingdom

New vaccines based on mRNA technology are emerging as an alternative to traditional vaccination to effectively respond to an epidemic event. One major advantage of these type of vaccines is that it allows to have a ready-to-produce vaccine in a matter of weeks. mRNA vaccines are produced in *in vitro* transcription (IVT) reactions that are catalysed by an RNA polymerase. This cell-free method delivers $g.L^{-1}$ of reaction in hours of reaction. However, to scale mRNA production in a cost-effective way requires a deep knowledge on the production process itself. IVT contains a large number of variables (e.g. enzyme, cofactor and substrate concentration) that can affect its outcome.

In this work we explore mRNA manufacturing process by optimizing the production process. In particular, we use Bayesian optimization, as a form of adaptive data-driven incremental design-of-experiments (DoE) to maximise mRNA production. We also use interpretability techniques, namely explanation models based on Shapley Values, to interpret the surrogate model predictions. Using this methodology, we were able to find optimal reaction conditions with 60 runs. In the end we obtain a maximum of 12 $g.L^{-1}$ total mRNA produced solely under 2 hours. This corresponds to a production increase of a factor of 2 in half of the time when compared to the best available baseline published industrial reaction. Additionally, the interpretability techniques allowed us to better understand the parameter's impact on the model, make a connection with existing literature, and ultimately increasing the understanding of IVT reactions for mRNA production.

O6 | Improving the reliability of oil spill identification by the accurate simulation of diagnostic ratios

Rocha, A.C. (1; 2); Palma, C. (1); Bettencourt da Silva, R.J.N. (2)

(1) Instituto Hidrográfico, Rua das Trinas, 49, 1249-093, Lisboa, Portugal;
(2) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências da Universidade de Lisboa, Ed. C8, Campo Grande, 1749-916, Lisboa, Portugal

In Analytical Chemistry, it is common to use statistical tests to assess the significance of measurement values. In general, it is assumed that the observed data of a given variable can be described by a normal probability distribution. The deviations from normality can affect the confidence intervals defined for the significance tests. Thus, this assumption is not always valid and can lead to errors in performed assessments. One way of dealing with this problem is estimating the confidence intervals by the exact distribution of probabilities of the variable under study. Here, the numerical simulation by the Monte Carlo Method (MCM) supported by experimental data, commonly used for solving deterministic or stochastic problems, proves to be an advantageous approach.

The aforementioned problem has been studied and applied in the oil spill source identification, which is obtained through chemical analyses performed on samples collected in the spill and suspected sources. The samples are analysed by Gas Chromatography-Mass Spectrometry, determining a wide range of oil-discriminating compounds. For each sample are determined ratios between compounds' abundances, i.e., diagnostic ratios (DR), which define the commonly named sample fingerprint. The oil spill source identification is obtained by comparing, statistically, the samples' fingerprints. Equivalence between the fingerprints of the spill and the suspected source samples is declared if, for all DR of the set that defines the fingerprint, is observed statistical equivalence. However, reference methodologies for oil spill identification [1; 2] have been using DR comparison methods based on approximations or on the normality assumption of DR probability distributions.

The present work intends to demonstrate the impact that normality deviations of the DR distributions have on the assessment of equivalence between DR and to present an innovative method for DR comparison, recently developed and based on simulation by MCM [3], which improves the oil spill identifications quality.

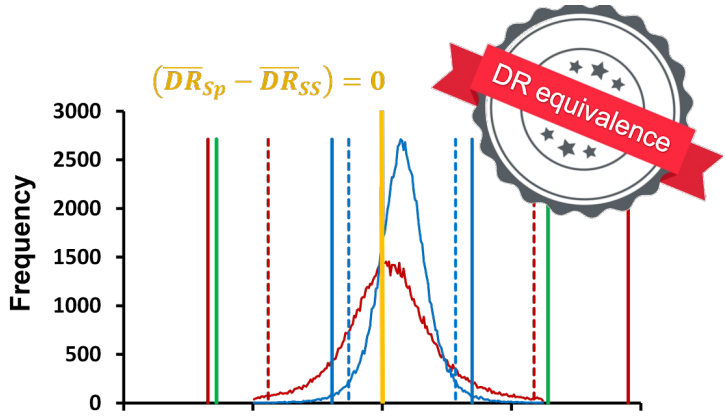


Figure 1: Simulated and modelled probability distributions of $((\overline{DR22})_{Sp} - (\overline{DR22})_{Ss})$ by the MCM (red) and St methods (blue), determined from triplicate injections of spill (Sp) and suspected source (SS) samples for ratio format AB. . . . P2.5 and P97.5; __P1 and P99; $(\overline{DR}_{Sp} - \overline{DR}_{Ss})$; __SCLL and SCUL (green) as criteria defined by CEN/Tr 15522-2 [2].

[1] Daling, P.S. et al. (2002) Environ. Forensics, 3, 263-278. ;
[2] CEN (2012). CEN/TR 15522 2:2012:E. Brussels Studies Institute. Brussels:Belgium. 138 p. ;
[3] Rocha, A.C. et al. (2022) Chemosphere, 289, 133085.

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O7 | Towards a framework to unify *in silico* methods for endocrine disruptors identification: a machine learning model to predict thyroid peroxidase inhibition

Calçada, Bruno (1,2), Sartini, Andrea (3), Fowkes, Adrian (3), Victor, Bruno L. (2), Costa, Paulo (2)

(1) Ascenza Agro S.A, Alameda dos Oceanos Lote 1.06.1.1 D - 2º, 1990-207 Lisbon,
(2) BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences of the University of Lisbon,
(3) Lhasa Limited, Granary Wharf House, 2 Canal Wharf, Leeds LS11 5PS, England

The increased incidence of endocrine-related human diseases due to a recurrent daily exposure to endocrine-disrupting chemicals (EDCs) has led to a growing interest in understanding how these chemicals can affect the human endocrine system. A request by the European Commission (EC), led to the issue of a guidance to identify this class of compounds in the context of Plant Protection Products (PPPs) and biocidal products [1]. Considering this guidance, the development of new *in silico* methodologies capable of identifying EDCs is of utmost importance, not only for drug discovery but also to support decision-making for chemical risk assessment.

In this communication, the initial development of a machine learning (ML) model, focused on thyroid pathways, and more specifically on the inhibition of thyroid peroxidase (TPO), will be discussed. TPO is of utmost importance in the regulation of multiple physiological processes of the endocrine system since it catalyses the iodination as well as the coupling of tyrosine residues to thyroglobulin to generate T3 and T4 thyroid hormones. The curation steps of a dataset assembled from a high-throughput *in vitro* assay developed in the Endocrine Disruptor Screening Program by the United States Environmental Agency to predict TPO inhibition via the oxidation of Amplex UltraRed in the AUR-TPO assay [2,3] and, the subsequent development of baseline ML models will be presented.

Our results will be compared with previously reported workflows applied to similar datasets and a discussion on the importance of the selected curation steps will be undertaken considering the obtained ML performance. The best ML model derived from our work will serve as a baseline for new ML improvements implemented in the future, which ultimately will allow for an accurate identification of endocrine disruptors.

[1] Andersson, N. et al., EFSA Journal 2018, 16, 5311
[2] Friedman, F.K., et al., Tox. Sci., 2016, 151, 160
[3] U.S. EPA, ToxCast & Tox21 Summary Files from invitrodv_v3.2, 2021
Available:https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

Funding: Project funding UIDB/04046/2020 and UIDP/04046/2020. Project reference 2021.09731.CPCA

O8 | Iron-cyclopentadienyl compounds with phosphane and *N*-based ligands show strong activity against a broad panel of human cancer cell lines

Pilon, Adhan (1); Garcia, M. Helena (1); Valente, Andreia(1)

(1) Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

Cancer is one of the deadliest diseases worldwide. Currently, platinum-based drugs are the most used chemotherapeutics, alone or with other drugs. However, many noxious side effects are associated to their use.[1] In that frame, the use of biologically essential metals, such as iron, seems a valuable strategy. Our group started to develop '$Fe(\eta^5-Cp)$' complexes as anti-cancer agents more than 10 years ago, which has become the focus of my PhD thesis. All families of compounds, designed having in view structure-activity studies, have the $Fe(\eta^5-Cp)^+$ fragment in common. The cationic families of *piano stool* iron-cyclopentadienyl complexes with the general formula $[Fe(\eta^5-Cp)(CO)(PR_3)(L)]^+$, where L = benzonitriles with different substituents(NCR) or imidazoles(Im-R), and PR_3= triphenylphosphane, 4-(diphenylphosphino)benzoic acid or tris(4-fluorophenyl) phosphane, have been developed with the main purpose of studying the effect that the different substituents or ligand had on the compounds' anticancer activity.[2] From all the tested phosphane-based ligands, PPh_3 was the one showing the best activity. Thus, it was decided to keep the fragment $Fe(\eta^5-Cp)(CO)PPh_3{}^+$ while changing the nature of L.

The biological activity for compounds with NCR was tested in two different tumor cell lines, breast MDA-MB-231 and colorectal SW480, and in the normal colon-derived cell line NCM460. All compounds were cytotoxic in the micromolar range, showing an intrinsic selectivity for the SW480 line(*vs.* NCM460). For the SW480 cell line the compounds induce cell death by apoptosis and inhibit proliferation by hindering the formation of colonies and affecting the cytoskeleton of cells. The compounds with Imi-R ligands were studied in colorectal cancer cells sensitive(Colo205) and resistant(Colo320) to doxorubicin(a drug in clinical use), revealing IC_{50} values in the low micromolar range.[3] Altogether, it was possible to conclude that, for all families of compounds, the hydroxyl substituent at the L ligand leads to the lowest cytotoxicity, while more lipophilic substituents originate the best anticancer compounds.

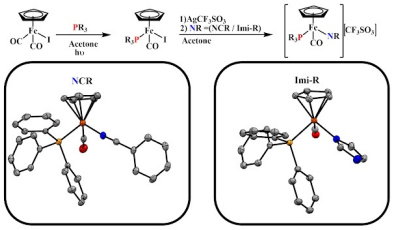

Figure 1: Scheme for synthesis of families with the general formula $[Fe(\eta^5-Cp)(CO)(PR_3)(L)]^+$.

[1] Gasser, G. et al. (2011) J. Med. Chem, vol. 54, pp. 3-25.
[2] Pilon, A. et al. (2017) J. Organomet. Chem., vol. 852, pp. 34-42.
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Flash
Communications

F1 | Development and simulation of a purification platform based to deliver clinical-grade mesenchymal stem/stromal cell-derived extracellular vesicles

Silva, Ricardo M. (1,2); Rosa, Sara Sousa (1); da Silva, Cláudia Lobato (1,2); Santos, José A. L.(1); Fernandes-Platzgummer, Ana (1,2) and Azevedo,Ana M. (1)

(1) Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
(2) The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Extracellular vesicles (EVs) are membrane vesicles fundamental to cell-cell communication through the exchange of their cargoes. For this reason, EVs have emerged as a new cell-free strategy with clinical applications for disease diagnosis and therapy. Taking EVs to the clinic requires an investment in the development of cost-effective manufacturing processes. So far, different downstream strategies have been implemented based on differential ultracentrifugation or PEG precipitation. These techniques, although efficient at lab-scale, might damage EVs integrity and originate protein aggregates which decreases the purity of final the product. Herein, we explore the implementation of a downstream platform composed of a nuclease digestion followed by anion exchange chromatography. Four different nucleases were evaluated, and their performance were optimized using a Design-of-Experiment approach. Chromatography allows targeting the product and separating it from impurities (host cell DNA and protein) in a scalable and cost-effective way.

We evaluated the performance of six AEC resins with different characteristics. All resins present a satisfying recovery ranging from 60% for Poros ® HQ, to 85% for Capto ™ Q ImpRes, while removing more than 98% of impurities. These results were integrated into an industrial-scale process simulated in SuperPro Designer. The designed plant has a capital investment of nearly 50 million €€ and an annual operating cost of 30 million €€. Without optimization 1406 clinical-grade doses of MSCs ($>1\times10^8$cells/dose) and 4902 clinical-grade doses MSC-EVs (2×10^{11} particles/dose) can be produced annually, and then can be sold for a competitive minimum selling price of 10800 €€ and 4015 €€, respectively. The simulated infrastructure shows promising economic indicators, with a payback time of 7 years and a net present value of 10.5 million €€. Concluding, we demonstrated that the downstream platform implemented delivers a product that meets the requirements imposed by regulatory agencies for phase I clinical trials. The industrial simulation shows its scalability, cost-effectiveness and viability.

[1] Li, X., Corbett, A. L., Little, J. P., et al. Challenges and opportunities in exosome research — Perspectives from biology, engineering, and cancer therapy. APL Bioeng. 3, 1–21 (2019) doi: 10.1063/1.5087122.

This work was funded by Fundação para a Ciência e a Tecnologia (FCT, Portugal) through projects PTDC/EQU-EQU/31651 /2017 and IF/00048/2014 and funding from iBB-Institute for Bioengineering and Biosciences (UIDB/04565/2020 and UIDP/04565/2020) and the Associate Laboratory i4HB-Institute for Health and Bioeconomy (LA/P/0140/2020). R.S. acknowledge a FCT PhD fellowship (UI/BD/151062/2021).

F2 | Electrochemical growth of Fe-MOF-74-type films

Reis, A. R. (1); Realista, S. (1); Gomes, C. S. B. (2) (3) (4); Viana, A. S. (1); Corregidor, V. (5); Alves, L. C. (5); Martinho, P. N. (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, 1749-016 Lisboa, Portugal;
(2) LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal;
(3) UCIBIO-Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal;
(4) Associate Laboratory i4HB-Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal;
(5) Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 km 139,7, 2695-066 Bobadela LRS, Portugal.

The fast development of new technological devices is highly related with the fabrication of thin films and/or membranes, which are often based on nanomaterials.[1] From all, metal-organic frameworks (MOFs) have shown some promising results that can be attributed to their extraordinary structural properties.[2] This work will focus on MOF-74 that with its characteristic crystalline structure, as well as the presence of highly reactive metal sites, are promising structures to interact with a variety of host molecules.[3]

Therefore, our group reports the synthesis of new ligands, characterised by nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FTIR). These will be applied to the electrochemical fabrication of Fe-MOF-74 and derivatives thereof, characterised by FTIR, scanning electron microscopy (SEM), atomic force microscopy (AFM), particle-induced X-ray emission (PIXE) and Rutherford backscattering spectrometry (RBS).

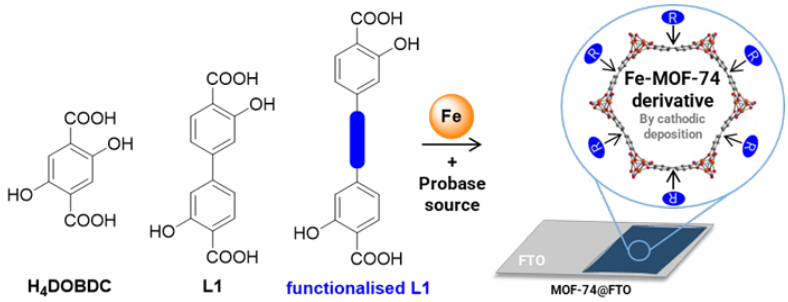

Figure 1: Electrochemical Fe-MOF-74-type film fabrication process.

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[2] Jia, Z. et al. (2020) Microporous Mesoporous Mater., 305, 110322.
[3] Easun, T. L. et al. (2017) Chem. Soc. Rev., 46, 239.

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F3 | Design, production and characterization of antiviral proteins targeting SARS-CoV-2

Parreiras, Susana (1); Cruz, Carlos H. (1); Valério, Mariana (1); Sousa, Pedro M. F. (2); Soares, Cláudio M. (1); Vicente, João B. (1) and Lousa, Diana (1)

(1) ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, www.itqb.unl.pt,
(2) iBET, Instituto de Biologia Experimental e Tecnológica, www.ibet.pt

SARS-CoV-2 virus is responsible for the current COVID-19 pandemic, which has caused >500 million infections and >6 million deaths (as of April 2022). Despite vaccination efforts, there remains an urgent need to develop strategies to control infection and treat patients. One of the proteins attached to the viral membrane is the spike (S) protein, that is primarily responsible for the virus' ability to enter host cells. It consists of two subunits: S1, containing a receptor-binding domain (RBD) responsible for binding to the host cell receptor, and S2, that facilitates the membrane fusion [1]. This makes it one of the most promising therapeutic targets.

The aim of this work was to design and produce antiviral proteins that can prevent the interaction between the S protein and the host receptor, angiotensin converting enzyme-2 (ACE2) protein, to block infection [2,3]. First, several antiviral proteins were computationally designed using the Rosetta program based on the interactions between ACE2 and the RBD. Next, six molecular dynamics simulations (MD) of 1 μs of three candidates were performed to test their interaction with the RBD. Followed by experimental validation of the three candidates. The secondary structure and thermostability of these proteins were tested by far-UV circular dichroism spectropolarimetry. Surface plasmon resonance was used to evaluate the affinity of each candidate for RBD. Neutralization assays were performed to investigate the neutralization ability of the proteins. The experimental results show that one of the developed proteins is a promising therapeutic approach that will be further improved in the future.

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[2] Cao L et al. (2020) Science 370(6515):426-431 ;
[3] Baig, MS et al. (2020) Drugs in R&D 20(3), 161-169

This work was supported by Fundação para a Ciência e Tecnologia (FCT) through the grant PTDC/CCI-BIO/28200/2017 and Project LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular) funded by FEDER and FCT, and LS4FUTURE Associated Laboratory (LA/P/0087/2020).

F4 | Use of Hansen Solubility Parameters (HSPs) to investigate the solubility behaviou of Hydrophilic and Hydrophobic NADES

Fernandes, Cláudio C. (1); Haghbakhsh, Reza (1); Paiva, Alexandre (1); Duarte, Ana Rita C. (1)

(1) LAQV, REQUIMTE, Departamento de Química, Nova School of Science and Technology, 2829-516 Caparica, Portugal.

Recently, a new generation of solvents, namely, Natural Deep Eutectic Systems (NADESs) are drawing special attention and interest of researchers because of their significant physicochemical properties such as biodegradability, low vapor pressure, easy synthesis, and good solubility. These solvents are formed by mixing a Hydrogen Bond Acceptor (HBA) and a Hydrogen Bond Donor (HBD) at different molar ratios, which leads to a compound with a lower melting point that its constituents. Nowadays, there are various applications that can benefit from NADESs, including the separation and extraction of various components. However, since the application of any solvent is depending on its intrinsic properties, it is crucial the knowledge of its characteristics. Hansen solubility parameters (HSP) are widely used in many scientific fields, mainly in pharmaceutical industry, where "solubility" is considered one of the most important parameters. Hansen theory's is based on the principle that "like dissolves like", which implies that two materials will only dissolve each other if they have similar HSP. Although Hansen book's [1] provides HSP of a wide range of pure compounds, unfortunately most of them are not NADESs and therefore cannot be used. However, authors such as Hoftyzer/Van-Krevelen [2], Stefanis/Panayiotou [3], Hoy [4] and even Hansen and Beerbower [5] developed some predictive models that can be used to estimate the HSP and consequently the miscibility/solubility of compounds.

Therefore, the main goal of this work consists in determining the HSPs of NADESs and some solutes (e.g., amino acids, drugs and vitamin), using those described models and then, validate them through the experimental tests of solubility. This work, will not only allow the choice of the most the suitable model to be used in NADESs field, as well as to improve it and finally to increase the knowledge on solubility of NADESs by having a comprehensive screening study.

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This work has received funding from the European Union's Horizon 2020 - European Research Council (ERC) - under grant agreement No ERC-2016-CoG 725034. This work was also supported by the Associate Laboratory for Green Chemistry (LAQV) which is financed by national funds from FCT/MCTES ((UIDB/50006/2020).

F5 | Studying glycerol permeability through aquaporin from *Plasmodium falciparum* for the development of new antimalarial therapies

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

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

Malaria is one of the largest public health problems and although most variants are successfully treated with the existing antimalarial drugs, this disease is still responsible for an outrageous 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 dislocation 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 a promising target for the development of new antimalarial therapies.

In this communication, we will present the first results of a multi-target strategy that couples keystone antimalarial drugs and PfAQP inhibitors. We will show results from Molecular Dynamics, Umbrella Sampling, and Potential of Mean Force calculations aiming at identifying structural determinants regulating the permeation of the natural subtracts across PfAQP. These results are of utmost importance for the next steps of the project where we will evaluate the inhibitory effect of multiple glycerol derivatives coupled with known antimalarial drugs on the PfAQP function.

[1] Phillips, MA et al. (2017) Nat Rev Dis Primers 3, 17050.
[2] Capela, R et al. (2011) Antimicrob. Agents Chemother. 55, 4698-4706.
[3] Dean P, et al. (2014) Front. Plant Sci. 5, 153.

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F6 | On the Role of Polyphenols in the Hydration and Aggregation of Parkinson's Disease Related Peptides

Nascimento, Catarina (1); Martins, Gabriel (1); Galamba, Nuno (1)

(1) Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal

Protein aggregation is implicated in several neurodegenerative diseases, including Parkinson's Disease (PD)[1]. PD's etiology has been associated with the formation of cytotoxic oligomers of which a-synuclein (α-syn), a 140 amino acid intrinsically disordered protein mainly expressed in the central nervous system, is the primary component. While several small molecules, including various polyphenols, have shown some aggregation inhibition activity *in vitro* there is still no approved drug that can disrupt and/or inhibit the formation of these aggregates in PD and other proteinopathies[2]. Furthermore, the action mechanism of these potential aggregation inhibitors remains poorly understood.

Herein, we study the effect of archetypal polyphenols in the solvation and binding of model peptides from the non-amyloid-β component (NAC), a highly hydrophobic and amyloidogenic domain of α-syn. These results are further compared with those for an 8M aqueous urea (a protein denaturant) solution, believed to disrupt hydrophobic interactions closely associated with the α-syn aggregation mechanism.

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[2] Stoker, T. B.; Barker, R. A. (2020) F1000Research, 9, 12

NG acknowledges financial support from Fundação para a Ciência e a Tecnologia of Portugal (CEEC/2018). GM acknowledges a PhD scholarship from Fundação para a Ciência e a Tecnologia of Portugal (2021.05348.BD). Work supported by UIDB/04046/2020 and UIDP/04046/2020 centre grants from FCT, Portugal (to BioISI).

F7 | Understanding the molecular basis for human mitochondrial glutamyl-tRNA synthetase (hERAS2) deficiency - from recombinant protein production to structural characterization

Lopes, Beatriz (1); Ribeiro, Joana V. (1); Hathazi, Denisa (2); Horvath, Rita (2); Gomes, Cláudio M. (1); Henriques, Bárbara J. (1)

(1) 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) Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, Cambridge, UK.

Mitochondrial diseases (MD) are a group of complex metabolic disorders defined by genetic defects that predominantly affect the mitochondrial oxidative phosphorylation pathway (OXPHOS) [1] due to the impairment of processes such as replication, transcription, or translation of mtDNA [2]. A group of enzymes that have been identified as key components of the mitochondrial translation apparatus are the mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) [2]. These enzymes are responsible for the addition of the corresponding amino acid into the correct tRNA molecule [2] during protein translation in the mitochondria. Recently, an increasing number of defects in mitochondrial protein synthesis, associated to mutations in mt-aaRS genes, have been identified. These deficiencies have been linked with diverse clinical presentations, mainly affecting the central nervous system.

Although reports of mutations in these enzymes seem to be increasing, there is still limited information regarding the structural and conformational consequences of disease-causing mutations. This project strives to fill that gap by employing a variety of biochemical and biophysical approaches to perform a structural and conformational characterization of the human mitochondrial glutamyl-tRNA synthetase (hEARS2). We have tested several conditions for recombinant protein expression in *E.coli*, including different growth temperatures, variable IPTG concentrations and co-expression with molecular chaperones. Our results indicate that the ideal condition for protein production was to induce hEARS2 expression using 0.5 mM IPTG and growing the cells at 22^{o}C overnight. The purification process was then optimized to include an IEX column to enrich the extract with the hEARS2 protein, prior to the affinity purification with the his-tag present on the enzyme. Afterwards, we used biophysical methods, such as circular dichroism, fluorescence spectroscopy or differential scanning fluorimetry (DSF), to perform detailed structural and conformational protein characterization.

Overall these studies bring important new information regarding hERAS2 recombinant production, and most importantly provide tools to proceed with our studies on hEARS2 disease variants.

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[2] Diodato, D et al. (2014) Int J Cell Biol.

F8 | Preparation of oxygenated metabolites of agrochemical active ingredients

Clemente, Duarte B. (1,2), Monteiro, Carlos M. (3), Coelho, Jaime A. S. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa;
(2) Department of Chemistry and Biochemistry, Faculdade de Ciências, Universidade de Lisboa;
(3) ASCENZA Agro, S.A., Screening & Synthesis Laboratory, Setúbal, Portugal

The development of plant protection products requires the safety profile analysis of active ingredients (AIs). This includes toxicity determination of AI metabolites. A very common phase-one metabolism reaction is C-oxygenation, catalyzed by cytochrome P450 enzymes.[1] Thus, the synthesis of oxygenated AI metabolites is of great importance to agrochemical producing companies, namely ASCENZA Agro, for safety evaluation purposes.

Herein, we describe the synthesis of hydroxylated aromatic and benzylic metabolites of several AIs, using methods described by Tobias Ritter and co-workers (Figure 1).[2,3] These methods allow the late-stage oxygenation of the aromatic and benzylic positions, by generating mesylate derivatives with bis(methanesulfonyl) peroxide as an oxidant, followed by conversion to the corresponding alcohols.

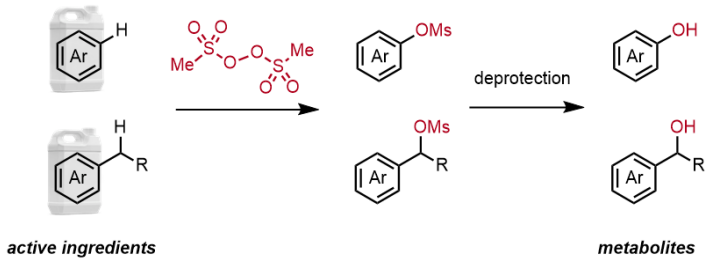

Figure 1: Synthetic route for the late-stage aromatic and benzylic oxygenation of AIs.

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[3] Ritter, T. et al. (2019) J. Am. Chem. Soc. 2019, 141 (45), 17983-17988

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F9 | Development of a machine learning-based pipeline able to predict genes associated with diseases and cell processes using interpretable network embeddings

Coelho, Alexandre (1), Pinto, Francisco R.

(1) BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

In the last decades, we have seen a huge growth in the number of know proteins. However, the functional mapping of proteins with cell processes and diseases is still incomplete, with only about 10 % of proteins having a known disease association [1]. Experimental methods can find these associations yet, are both slow and expensive. Computational methods thereby emerge as an alternative for the prediction of these associations, with many relying on protein-protein interactions (PPI) networks and proximity/connectivity metrics [2, 3]. However, these algorithms only rely on the information of the disease/process they are trying to predict, which might not have enough quality.

Here we propose to develop a network-based prediction algorithm able to find new gene-disease associations based on the information of additional relevant processes and diseases. Our pipeline was evaluated across several network conditions for the classification of 429 cell processes and 301 diseases. Results show a significant increase in the prediction capability of the proximity/connectivity metrics used, with the Random-Walks with Restart showing an f-measure score consistently above 0.9. Validation of the new predictions is still required, such as the analysis of the protein's known phenotypes and connection to the disease ones. Nonetheless, preliminary results show that this pipeline will serve as a more complete and robust prediction tool, that will lead to a better understanding of specific processes and diseases, with the possible discovery of new therapeutic targets.

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I thank Professor Francisco Pinto for his guidance throughout the project. A special thanks to BioISI and Fundação para a Ciência e a Tecnologia for funding this project with a fellowship (BII) under the BioISI's Junior Programme.

F10 | Tailoring polydopamine and polynorepinephrine coatings for bacterial laccase based phenolic biosensor

Almeida, Luís C. (1); Branco, Clara (1), Moreira, Helena (1), Morana, Alessandra (2); Squillaci, Giuseppe (2); Ihalainen, Petri (3), Sobhana, Liji (3); Correia, Jorge P. (1); Viana, Ana S. (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências da Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal;
(2) Research Institute on Terrestrial Ecosystems (IRET), National Research Council of Italy, (CNR), Via P. Castellino 111, 80131 Naples, Italy;
(3) MetGen, Rakentajantie 26, 20780 Kaarina, Finland.

Thermophilic bacterial laccases are promising biocatalyst for biosensing, since they are active in a wider range of pH and less susceptible to inhibitory agents compared to the eukaryotic laccases extracted from fungi and plants. However, bacterial laccases are typically low redox potential enzymes which discourages their study and application in electrochemical devices. Notwithstanding, modified electrodes with Laccase from *B. subtilis* have already shown superior catalytic properties, provided enhanced sensor stability and reusability than usual fungal laccases [1]. On the other hand, bio-inspired polydopamine (PDA) became a reference material in surface modification due to its simple chemical preparation on virtually any substrate. The catechol groups found in PDA display important reactivity towards nucleophilic groups (through Michael-type addition or Schiff's base formation), allowing the attachment of biomolecules to electrode surfaces. Our recent work demonstrated the suitability of tailored PDA coatings as bioconjugation platforms for affinity and enzymatic sensors [2,3]. PDA is by far the most acquainted catechol-derived coating; however, other promising catecholamines, possessing additional chemical groups, whose synthesis and physicochemical properties are not yet satisfactorily explored.

Hereby, we carried out a comparative study between the performance of two electrosynthesized polymers, PDA and polynorepinephrine (PNE), as transducing matrices in amperometric biosensors. We demonstrated that thin PNE films enable the electron transfer of anionic and cationic species at pH 5 and 7, whereas PDA with a similar thickness inhibit charge transfer of anionic species at pH 7 and cationic species at pH 5. This phenomenon denotes the influence of polymer charge at working pH which may limit the application of PDA-based platforms. Furthermore, the two polymers were successfully bioconjugated with a bacterial laccase, and their catalytic performance demonstrated, at neutral pH, towards simple phenols (gallic acid) and flavonoids (catechin). PNE stands out as an alternative bio-inspired material for electrochemical biosensing applications.

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Fundação para a Ciência e a Tecnologia through PhD scholarship SFRH/BD/129566/2017, COVID/BD/152149/2022, projects UIDB/00100/2020 and UIDP/00100/2020; and Bio Based Industries Join Undertaking under the European Union's Horizon 2020 research and innovation program under grant agreement No. 792061 (SWEETWOODS).

Poster

Communications

P1 | The Energetics of Caffeine Polymorphs

Gilberto, G.M.(1); Bernardes, Carlos E. S. (1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa

The study of crystalline structures of a substance and their physical properties is an important field of research with applications in various areas such as the development of new medicines, explosives, paints, and electronics [1]. This is related to the ability of a substance to exhibit polymorphism (i.e., a substance that can form materials with distinct molecular packings), which results in crystal phases that can have significantly different physical properties (e.g., color, melting temperature, and solubility). Therefore, the prediction of these properties is of the utmost importance in designing materials with optimal properties for a given application.

Promising methods to achieve this goal include the use of computational techniques, like molecular dynamics simulations. These are based on intermolecular force fields, which, employing simple atom-atom interaction potentials, can be used to predict many properties of materials. Nevertheless, due to the empirical nature of these force fields, they need to be validated against benchmark experimental data, like molar enthalpies of sublimation (δsubHo) and crystal structure unit cell parameters [2]. This type of results can be found in the literature. However, δsubHo values that can be safely assigned to specific polymorphs are rarely found. Hence, the development of a benchmark database of enthalpies of sublimation for specific crystal structures of materials is a crucial step for the development of theoretical methods. This work aims at the revaluation of the enthalpy of sublimation of both polymorphs of anhydrous caffeine (figure 1) to obtain a benchmark value that can resolve the 10 kJ.mol^{-1} discrepancy that exists between values in the literature [3]. The experimental measurement was performed by Calvet microcalorimetry using samples characterized by powder X-ray diffraction and differential scanning calorimetry.

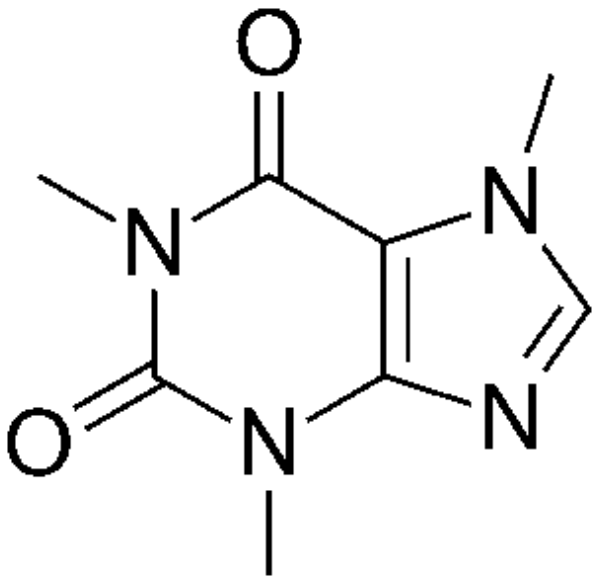

Figure 1. Molecular structure of caffeine.

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P2 | In Silico identification and Synthesis of C-MYC G4 and Helicase Interaction Inhibitors

Aljnadi, I(1,2); Paulo, A(1), and Victor, B L(2)

(1) MedOrgChem@iMed, Faculdade de Farmácia, Universidade de Lisboa.
(2) BioISI Faculdade de Ciências, Universidade de Lisboa.

G-quadruplexes (G4) are four-stranded nucleic acid secondary structures formed by guanine-rich sequences of DNA or RNA. G4s are involved in relevant biological functions of mammalian cells but, more importantly, they are over represented in cancer cells. Studies have found G4 in telomeres and promotor regions of several oncogenes, including c-MYC, which plays an important role in cellular regulatory processes as well as in cancer development and progression[1].

G4 formed in the promoter region of c-MYC may constitute an anticancer drug target by inhibiting DNA transcription via blocking the binding of transcription factors. Interestingly, G4 in the c-MYC promoter is reported to be unwounded by the helicase DHX36, a protein of the eukaryotic DEAH/RHA family that specifically recognizes G4s. Therefore, we have used the recently published crystallographic structure of the DHX36 helicase complexed with the c-MYC G4 to develop potential c-MYC G4-DHX36 interaction inhibitors as a novel class of anticancer drugs[2]. Starting from an initial library of 1104 indoloisoquinoline (IDQ) derivatives, we performed a molecular docking screening campaign to identify the most promising c-MYC G4 binders. From the Molecular Docking studies, we selected 20 compounds. The chemical synthesis of this subset of IDQ ligands was then started, by exploring the optimal reaction conditions. After the successful synthesis of these compounds, additional *in vitro* assays for c-MYC: G4-helicase interaction inhibitors will be enrolled to validate the computational predictions.

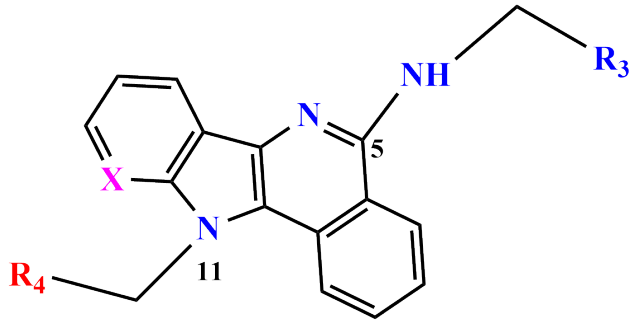

Figure 1: Chemical structure of indoloisoquinoline derivatives

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P3 | Metabolic Profiling of Extracellular Vesicles from Ascitic Fluids of Ovarian Cancer Patients

Trindade, Gonçalo (1,2); Mendes, Rita (1,2); Muzaferovic, Ines (1,2); Rodrigues, Leandra (1,2); Silva, Fernanda (3); Guerreiro, Ana C. L. (1,2); Silva, Sandra D. (1,2); Gomes-Alves, Patrícia (1,2); Félix, Ana (3,4); Alves, Paula M. (1,2); Brito, Catarina (1,2); Isidro, Inês A (1,2)

(1) iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
(2) Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
(3) CEDOC, Centro de Estudo de Doenças Crónicas, Nova Medical School, Universidade Nova de Lisboa, Lisboa, Portugal
(4) IPOLFG, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal

Ovarian cancer (OvC) is one of the deadliest cancers in women, characterized by advanced stage at diagnosis, high therapy resistance and tumor recurrence. Uncovering new biomarkers is thus of utmost importance for early diagnosis, choice of therapy and monitoring of disease progression. At the symptomatic stage, most women present ascites, malignant accumulation of ascitic fluid (AF) in the peritoneal cavity. AF is enriched in cells and their secreted products, such as extracellular vesicles (EVs). EVs have been attracting attention as versatile cell-cell communication mediators, thus gaining interest as potential circulating biomarkers in liquid biopsies, offering a portrait of the tumor proteins, nucleic acids, lipids and metabolites. Most studies involving EVs are focused on their RNA and protein cargo. Therefore, the metabolome is the least researched component of EVs. However, characterization of the metabolite content is both useful for clinical applications and to elucidate biochemical pathways and mechanisms of action of EVs within the cancer context.

Here, we assess the feasibility of metabolic profiling of EVs derived from AF and peritoneal washes (PW) of OvC patients as a source of prognostic biomarkers. We successfully isolated EVs from OvC patient biofluids which were characterized by enrichment analysis of typical vesicle protein markers, size, and morphology. Targeted LC-MS metabolomics and unsupervised analysis revealed distinct profiles of AF and derived EVs which identify networks correlated with the TCA cycle, regulation of DNA and histone methylation, and mediation of redox processes balance. might be used as a platform to generate new insights into underlying mechanisms of disease progression and metastasis in OvC, which may contribute to uncovering drug targets and potential biomarkers predictive for stratification, prognosis, and prediction of therapy response, thus supporting future applications in precision medicine. Ultimately, this study opens avenues for uncovering drug targets and potential biomarkers to monitor the treatment response.

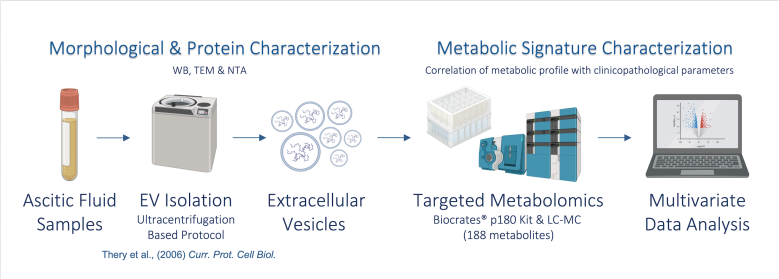

Figure 1: Graphical Abstract: Platform for the isolation of extracellular vesicles from ascitic fluid of ovarian cancer patients and metabolic signature characterization.

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P4 | Metal Complexes of Schiff Bases and Reduced Schiff Bases Developed for Anticancer Therapy

Dias Machado, Mariana(1); Tomaz, Ana Isabel(1); Correia, Isabel(2)

(1) CQE - IMS, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
(2) CQE - IMS, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal.

Cancer encompasses a family of diseases consisting of abnormal cell growth with the potential to invade any part of the body. In 2020, it was considered a leading cause of death, with almost 10 million deaths worldwide. With such concerning statistics, the search for effective and safe treatments is of uttermost importance. Chemotherapy remains one of the most used treatments, particularly due to the success of cisplatin. Nevertheless, platinum compounds exhibit copious side effects, limited activity and acquired resistance [1]. Therefore, research on new agents with improved efficiency and better tolerance is now an intense field of investigation. Compounds bearing alternative transition metal ions have been regarded as possible drug candidates. Ruthenium complexes have shown great potential, due to their proven anticancer properties and the ability to overcome cisplatin's resistance [1]. Furthermore, iron complexes have also proven to be interesting with reported promising cytotoxic effect, aligning with the benefits of using an endogenous metal, namely an expected lower systemic toxicity [2].

The ligands binding the metal are of equal importance and several studies have highlighted the therapeutic action of Schiff base metal complexes. Schiff base ligands provide numerous advantages, mainly their ease of preparation, their robustness, the ability to coordinate with almost any metal and their anticancer properties [3]. Of the several compounds reported, complexes of $Fe(III)$ [2] and $Ru(II/III)$ [1] with Schiff base ligands are still scarce and roughly unexplored. In this work, we focus on the synthesis of metal complexes [$Fe(III)$ and $Ru(III)$] bearing Schiff base and reduced Schiff base ligands with the goal to evaluate both the impact of different metal ions and of small changes in the ligand structure on the cytotoxic activity/selectivity of the compound, and on the overall biological response of the complex.

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P5 | New potential antitubercular drugs through derivatization of weak acids

Antunes, Duarte(1); Pedro Pais, João (1); Pires, David (1); Anes, Elsa(1,2); Constantino, Luís(1,2)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

(2) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Developing new drugs is essential to counter human tuberculosis (TB) epidemic. Alongside HIV co-infection, multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains are decreasing the effectiveness of first and second-line TB drugs. The World Health Organization (WHO) registered 1.5 million fatalities caused by TB in 2020, where 14% were HIV co-infected patients [1].

Studies reported that certain weak acids, such as trans cinnamic and salicylic acids have anti-mycobacterial activity. It has been proved that for some acids, ester prodrugs are more active and can be activated by esterases to liberate the acids [2]. We synthesized cinnamic and salicylic acid derivatives containing nitro substituents in the aromatic ring and compared the activity of the compounds with the non-nitro containing compounds.

Cinnamic and salicylic esters were synthesized via two different methodologies: 1) Fischer esterification between the corresponding acids and desired alcohols (butanol; hexanol; octanol; decanol; dodecanol) using a catalytic amount of sulfuric acid; 2) addition of thionyl chloride to the weak acid to create the corresponding acyl chloride; followed by nucleophilic addition of the corresponding alcohol to the chloride, in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine; or potassium carbonate. The same nucleophilic addition methodology was used to synthesize cinnamic and salicylic amides with the corresponding amines. Minimum inhibitory and minimum bactericidal concentrations for the compounds against M. tuberculosis were obtained. Results will be discussed.

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P6 | Understanding Metastasis Organotropism Patterns Through Within-cell and Between-cells Molecular Interaction Networks

Miranda, João (1), Pinto, Francisco R. (1) BioISI—Instituto de Biossistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

Metastasis is the leading cause of cancer-related deaths (about 90%). Metastatic tumours develop when cancer cells disseminate from the sites of primary tumour growth and colonize distant organ sites [1]. Distant metastasis follows a non-random distribution among distant organs, known as "organotropism" or "organ-specific metastasis". Different cancer types and subtypes display distinct patterns of organotropism. These insights led Steven Paget to propose the "seed and soil" hypothesis, which suggests that cancer cell (seeds) have intrinsic compatibilities with particular organ microenvironments (soil) [2]. Recent studies support the notion that organ-specific metastasis depends not only on extrinsic factors enabling cancer cells access to organs, but also on their intrinsic abilities to interact with the host microenvironment [1].

Network medicine is the application of network-based approaches to characterize human disease. Most cellular components perform their functions through interactions with other cellular components. This interconnectivity implies that the impact of a specific genetic abnormality is not restricted to the activity of the gene product that carries it but can spread along the links of the network and alter the activity of seemingly unaffected gene products [3]. In this project, we will use a Systems Biology approach to study metastasis organotropism. We will build tissue-specific inter- and intracellular protein-protein interactions (PPI) networks between pairs of primary tumour tissue-metastasis site tissue and compute proximity/connectivity metrics between known tissue specific cancer driver genes and intercellular interactions. We hope to identify molecular determinants of tumour cell survival at specific organ microenvironments.

Our preliminary analysis of intercellular interactions PPI networks suggests that organotropism pairs of tissues tend to establish a higher number of intercellular interactions than control pairs, i.e., pairs with uncommon sites of metastasis. The main goal going forward is to identify common and specific interactions and determinants at play in metastasis development and organotropism.

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I thank my thesis supervisor, professor Francisco Pinto, for proposing this very interesting thesis project, and for his continued support, ideas, and insights.

P7 | Counter-Ion Effect In The Magnetic Properties of Fe(III) X-Br-SalEen Complexes

de Lemos, Ricardo J. (1); Marques, Rafaela T. (1); P. Ferreira, Liliana (2) (3); Martinho, Paulo N. (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, 1749-016 Lisboa, Portugal.
(2) BioISI - Biosystems & Integrative Sciences Institute, University of Lisboa, Faculty of Sciences, Campo Grande, C8, 1749-016 Lisboa, Portugal.
(3) Department of Physics, University of Coimbra, 3004-516 Coimbra, Portugal

In the last few decades, materials and complexes exhibiting spin crossover (SCO) have sparked quite a bit of interest, due to their possible applications as memory devices, sensors and/or switches, utilizing their magnetic, optical, or luminescent responses to temperature, pressure, or even both [1]. However, designing these complexes is still a great challenge [2]. As such, in this study, we have varied the position of a -Br substituent on the aromatic ring portion of our SalEen ligand, formed $Fe(III)$ complexes and used different counter ions for each -Br compound, with hopes of finding a correlation between ligands and the magnetic behaviour.

To achieve this, the ligand was synthesized by reacting the starting X-Br-salicylaldehyde with N-ethylethylenediamine, then adding different salts, in combination with $FeCl_2$, to obtain the various counter-ions (ClO_4^-, BF_4^-, PF_6^-, NO_3^-, Cl^-, OTf^-, I^- and BPh_4^-). The complexes have been characterized by SQUID magnetometry, FTIR and UV-vis spectroscopy. The compounds have shown a wide range of magnetic profiles, some displaying hysteresis loops and others gradual and complete SCO (Figure 1).

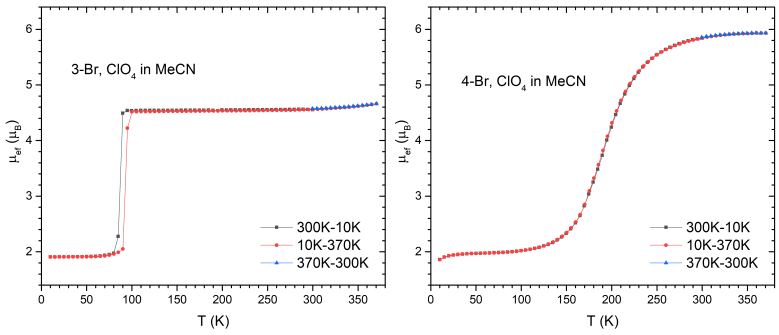

Figure 1: Comparison between X=3 and X=4's magnetic profiles for $[Fe(X\text{-}Br\text{-}SalEen)^2]ClO_4$.

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P8 | Production of the SARS-CoV-2 Spike protein and its Receptor Binding Domain (RBD) in Medicago cell suspension cultures

Ferreira, Ana Clara (1); Rebelo, Bárbara A. (1); Abranches, Rita (1)

(1) Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

The COVID-19 pandemic, caused by the worldwide spread of SARS-CoV-2, has prompted the scientific community to rapidly develop efficient and specific diagnostics and therapeutics. A number of avenues have been explored, including the manufacture of COVID-related proteins to be used as reagents for diagnostics or treatment. In eukaryotic cells, predominantly in mammalian cell cultures, the stable production of ACE2, RBD, and Spike proteins was successfully achieved, contrary to what is known for the microbial systems. In plants, COVID-related proteins were transiently expressed mainly in Nicotiana benthamiana. However, stable production of these proteins is yet to be achieved in a plant-based system. In this work, a legume plant model platform was used as an alternative eukaryotic system for the production of SARS-CoV-2 Spike glycoprotein and RBD.

To achieve this goal, we used Medicago truncatula A17 cell suspension cultures, which were engineered to stably produce the full-length His-tagged-RBD and -Spike recombinant proteins. Protein analysis of the culture media, of each transformed culture, showed the presence of the Spike or RBD proteins with different glycoforms. Both proteins are continuously and stably being produced by Medicago cells and the purification process for both proteins is ongoing using chromatographic methods. The following step will be an evaluation of the trimeric Spike protein in the culture medium, strengthening the potential of plant cell cultures as production systems for large, complex proteins.

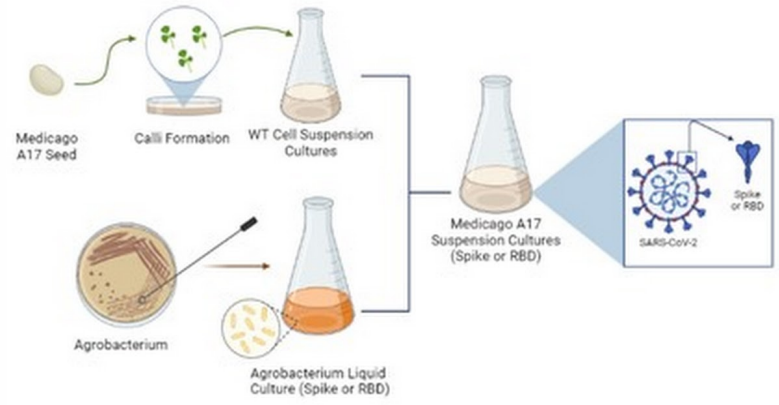

Figure 1: Graphical Abstract

P9 | Electroreduction of CO2: Fe(III) salphen complex as catalyst

Marques, Rafaela T.;(1) Realista, Sara;(1) Martinho, Paulo N.(1)

(1) Centro de Química Estrutural - Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa.

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 (CO and H_2) or directly to liquid fuels (methanol and/or methane).

The electroconversion 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. The $Fe(III)$ complexes are known for being good catalysts and the synthesis with salphen ($N, N' - bis(salicyldene) - 1, 2 - phenylenediamine$) ligands is easy and with high yields. Therefore, the synthesis and characterisation of a mononuclear $Fe(III)$ saphen complex is reported. Cyclic voltammetry, spectroelectrochemical and electrocatalyst studies of the complex are also investigated to be used as catalysts.

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P10 | An ad hoc HTVS protocol to identify new CRM1 non-covalent inhibitors

G. N. Sequeira, João (1); Link, Wolfgang (2); Machuqueiro, Miguel (1)

(1) BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM). 28029, Madrid, Spain

Protein function is coupled to its subcellular localization, since it restricts the access to binding partners and enzymes that catalyze post-translational modifications. The best-studied export protein is a transversal protein across all eukaryotic cells – the Chromosome Region Maintenance 1 (CRM1, also known as XPO1 or exportin 1). Being a nuclear exporter, CRM1 inhibition has long been idealized for the treatment of cancer and several viruses and it consists of binding a compound to the NES-binding groove to prevent the association of CRM1 with its cargo [1]. However, all known CRM1 inhibitors establish a covalent bond with Cys528, resulting in high toxicities and impairing its *in vivo* application [2].

With the intent of discovering non-covalent inhibitors of CRM1, we developed a high-throughput virtual screening (HTVS) protocol to be implemented using a database provided by our collaborator, Prof. Romano Silvestri (Head of Medicinal Chemistry, Sapienza Univ., Italy). To increase the probability of success of this protocol, we first needed to obtain representative conformations of the CRM1's apo-structure. We used MD simulations to sample the conformational landscape of the protein in water and developed an ad hoc protocol to select more representative apo-structures. This led to 2 conformations with an apparent "ready-to-bind" NES binding groove, which were used in a HTVS protocol. Many of the promising compounds selected from our molecular docking protocol were already tested experimentally by our collaborator, Professor Wolfgang Link (University of Madrid, Spain), still without success, but the search continues. In this work, I will share many of the details of our approach and what is now being done to increase the chances of identifying new non-covalent CRM1 inhibitors.

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P11 | The impact of SARS-CoV-2 Omicron variant on the human ACE2 binding: a computational study

Teixeira, Rita(1); Valério, Mariana(1); Borges-Araújo, Luís(1); Melo, Manuel N.(1); Vicente, João B.(1); Lousa, Diana(1); Soares, Cláudio M. (1)

(1) Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Lisbon, Portugal

Severe acute respiratory syndrome coronavirus 2 (SARS¬CoV-2) is the causative agent of the COVID-19 pandemic, which escalated into a global pandemic in early 2020, accounting for >500 million infections and >6.2 million confirmed deaths worldwide (as of 2022/05/27). The SARS-CoV-2 mechanism of transmission and infection involves the binding of the virus to the angiotensin-converting enzyme 2 (ACE2) host receptor through the receptor-binding domain (RBD) of the spike (S) protein. The RBD is a privileged target of our immune system and antiviral therapies. Throughout last year multiple vaccines and new therapeutics against SARS-CoV-2 have been developed. However, their effectiveness is challenged by the continuous evolution of SARS-CoV-2, accompanying the origin and spread of new variants of concern (VOC): Alpha, Beta, Gamma, Delta, and recently, Omicron. Among the reported mutations in the VOC S proteins, several are specific to the RBD, which are associated with higher transmissibility or the ability to escape the immune response of previously infected patients. [1] In late 2021, the newly SARS-CoV-2 Omicron VOC raised considerable global concern due to the presence of more than 30 mutations in the S protein, 15 of which occur in the RBD. Moreover, the newly Omicron lineages, BA.2, BA.4 and BA.5, showed a potential higher transmissibility.[2,3]

Here we investigated the impact of the VOC RBD mutations on its interaction with ACE2, with a major focus on the Omicron RBDs, by performing microsecond molecular dynamics (MD) simulations of theses complexes. Our analysis of the binding and structural dynamics of these mutations provided a detailed characterization of the binding mode between the Omicron RBDs and the receptor. This allowed us to understand the role of key residues in the Omicron RBD-ACE2 interface and the effect of specific substitutions on the binding affinity via the establishment of new inter¬protein contacts.

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P12 | A metabolomics approach to the study of the effects of single-gene deletions in yeast

Luz, João (1); Pendão, Ana Sofia (1); Traquete, Francisco (1); Ferreira, António E.N (1); Sousa Silva, Marta (1); Cordeiro, Carlos (1)

(1) Laboratório de FTICR e Espectrometria de Massa Estrutural, MARE-Marine and Environmental Sciences Centre,

Saccharomyces cerevisiae , also known as baker's yeast, is a model eukaryote with around 6000 genes. Most of these genes can be deleted without compromising cellular viability, and a vast fraction of them without producing noticeable changes in the phenotype. Nevertheless, the mutated cells may differ substantially from their wildtype counterparts at the metabolome level, particularly if the mutations in question happen to be associated with key metabolic pathways. High resolution analytic techniques such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) allow us to identify and interpret these differences through an untargeted metabolomics approach.

In this work, we demonstrate an application of this paradigm to five isogenic *S. cerevisiae* strains. Besides the wild-type strain, we chose three null mutants involved in the methylglyoxal catabolism, lacking the genes encoding the glyoxalase I, glyoxalase II and aldose reductase catalytic enzymes. Another strain, lacking the enolase 1 gene, related to glycolysis, was also analysed. All strains were grown under the same conditions, without any alteration in growth phenotype being reported. Afterwards, metabolite extraction was performed and the extracts were analysed through FT-ICR-MS. The identified metabolites were putatively annotated with names (using human and yeast metabolomic databases as reference) and chemical formulas (predicted based on a set of heuristic rules).

Through the application of multivariate statistical analysis techniques (PCA, hierarchical clustering, and PLS-DA), we were able to show that it was possible to distinguish between the five isogenic strains based on their metabolic profiles. Furthermore, it was possible to explain some of the observed metabolic differences based on our pre-existing knowledge of methylglyoxal catabolism (e.g a greater degree of similarity was found between mutant strains related to the glutathione-dependent pathway of methylglyoxal catabolism), showing that biologically meaningful conclusion can be drawn from the study of single-gene deletions at the metabolome level.

P13 | *In silico* approaches to study membrane permeability of new antitumor Ru compounds

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

(1) BioISI - Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa
(2) Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa

Cancer has become one of leading causes of death around the globe, with female breast cancer as one of the most prevalent. Triple-negative breast cancer (TNBC) - lacking expression of estrogen and progesterone receptors and human epidermal growth factor receptor 2 - is among the subtypes with higher aggressiveness, having a notably poor prognosis[1]. TNBC lacks targeted therapies and presents heterogeneous responses to treatment with traditional cisplatin-like drugs, in part due to the development of multidrug resistance (MDR). TM34 $[RuCp(PPh_3)(2,2'-bipy)][CF_3SO_3]$ (where Cp = η^5-cyclopentadienyl, 2,2-bipy = 2,2'-bipyridine and PPh_3 = triphenylphosphane) is a Ruthenium-based compound that has been suggested to be a more efficient and selective therapy agent than cisplatin[2]. More recently, our colaborator Tânia S. Morais has been developing new derivatives of TM34 by adding peptide sequences that are recognized by receptor proteins from the FGFR family (overexpressed in TNBC)[3] and a pH-sensitive linker (targeting the lower pH of the tumour microenvironment), increasing the compound's selectivity.

The main goal of this work is to study the interaction of TM34, and several promising derivatives, with a membrane model (POPC) and to calculate their membrane crossing energy profiles, that can be used to estimate their membrane permeability coefficients. Using Molecular Dynamics methods, we can study the molecule's unrestrained partitioning into the membrane. This allows the identification of the preferential insertion region and orientation for these compounds. Additionally, by implementing an enhanced sampling scheme, like Umbrella-Sampling, we can simulate the compounds at various insertion depths. With this approach, we can obtain the potential of mean force profiles, allowing the calculation of the membrane permeability using the inhomogeneous solubility-diffusion model[4].

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P14 | Synthesis of iron complexes for applications as magnetic sensing materials

Gomes, Tiago (1), Oliveira, Bárbara (1), Ferreira, Liliana (2, 3), Xavier, Nuno (1), Martinho, Paulo (1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, 1749-016 Lisboa, Portugal;
(2) Biosystems and Integrative Sciences Institute (BioISI), Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa, 1749-016, Portugal;
(3) Department of Physics, University of Coimbra, 3004-516 Coimbra, Portugal

There is a great interest in the study of magnetic systems capable of combining their spin lability with the effect of optical rotation, under the main topic of magnetic dichroism. Said phenomena happens when a molecule is exposed to unpolarized and polarized light beams, resultant of the combined effect of its optical rotation and magnetic behavior, which can show promising applications as magnetochiral molecular sensors [1].

In this communication, we report the synthesis and characterization of bidentate and tridentate ligands, shown in Figure 1, as well as the magnetic properties of their Fe(II) and Fe(III) complexes. While the Schiff-base ligand preferably generates discreet Fe(III) complexes, the other two ligands are prone to form Fe(II) coordination polymers [2,3].

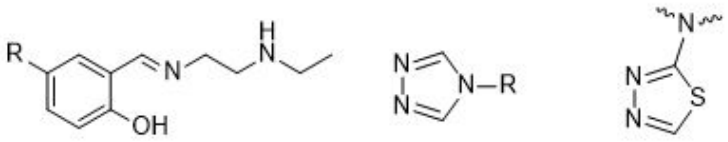

Figure 1: Structures of the organic ligands used in the synthesis of Fe(II) and Fe(III) compounds.

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P15 | Exploring the role of HOXA9 in IL7R-mediated B-cell leukemogenesis

Gama, Sara (1); Duque, Mafalda (1); Barata, João T. (1)

1. Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade Lisboa

B-cell acute lymphoblastic leukemia (B-ALL) is a hematological malignancy that arises from the clonal expansion of B-cell precursors. IL-7/IL-7R signaling is essential for the development and maintenance of the lymphoid compartment.1 In the past, our lab has described gain-of-function mutations in IL7R that leads to the constitutive activation IL-7R-mediated signaling. 2 Recently, we generated and characterized a conditional knock-in mouse model, expressing the IL7R mutation under the control of the endogenous promoter, which develops leukemia resembling PAX P80R or Ph-like precursor B-ALL.3 RNA-seq analysis of these leukemias revealed a significant downregulation of HoxA9 levels in leukemic samples when compared with normal B-cell precursors. This was puzzling, since HoxA9 plays an important role in hematopoiesis and leukemia. In contrast, our data suggests HoxA9 may act as a tumor suppressor in most B-ALL subtypes, including IL7R-dependent cases. Preliminary results in mouse primary leukemia cells overexpressing HoxA9 demonstrated decreased leukemic cell fitness *in vitro* and *in vivo* , suggesting that IL7R mutant leukemias with low HoxA9 levels have a selective advantage. At the molecular level, HoxA9 overexpression in IL7R mutant leukemias associated with decreased activation of IL-7R downstream pathways (JAK/STAT, PI3K/AKT and MEK/ERK).

To confirm the anti-leukemia role of HoxA9 in these leukemias, we intend to perform a knockdown of this gene in pre-leukemic cells to understand if it increases cell fitness and/or accelerates leukemia onset. Additionally, we aim to understand whether epigenetic mechanisms are involved in HoxA9 downregulation in leukemia cells. Using a histone deacetylase inhibitor and a DNA methyltransferase inhibitor we will evaluate the impact of acetylation and methylation on HoxA9 mRNA levels. Overall, our goals are to demonstrate that HoxA9 downregulation accelerates IL-7R-mediated B-ALL development and to understand how such downregulation occurs.

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P16 | Design and synthesis of halogenated glycomimetics targeting carbohydrate-binding proteins

Nunes, Rafael (1,2); Costa, Paulo J.(2); Xavier, Nuno M.
(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa;
(2) Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa

The design of glycomimetic structures is a common strategy in chemical glycobiology and carbohydrate-based drug discovery given the ubiquitous role of carbohydrates in biological recognition processes and hence their medicinal interest.[1] In this context, a variety of functionalities are often incorporated in carbohydrate templates1 whereas the utility of heavier halogens (X = Cl, Br, I) has been largely neglected. These species are able to establish specific interactions with Lewis bases, known as halogen bonds (XBs), through an electrophilic region at the tip of the halogen, known as σ–hole.[2] These interactions have found varied applications across the chemical sciences, including in medicinal chemistry.[3]

In this work, we synthetized a series of glycosides bearing halogen substituents as novel chemical probes to investigate the potential of XBs to act as surrogates for intermolecular interactions commonly involved in the molecular recognition of carbohydrates by proteins. Target structures include aryl galactopyranosides halogenated at the primary position that were rationally designed taking advantage of DFT calculations in order to establish their relative potency as XB donors. These glycomimetic entities will serve as tools to study protein–carbohydrate binding and will ultimately be screened as inhibitors of galactophilic lectins in the search for novel bioactive molecules targeting pathogenic bacteria.

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P17 | Identification, design and synthesis of novel indoloisoquinolines inhibitors of c-myc:G4-helicase interaction

Bahls, Bárbara (1,2); Paulo, Alexandra (2); Victor, Bruno L. (1)

(1) BioISI Faculdade de Ciências, Universidade de Lisboa
(2) MedOrgChem@iMed, Faculdade de Farmácia, Universidade de Lisboa.

G-quadruplexes (G4) are a noncanonical higher-order structure formed in guanine rich DNA or RNA sequences. They can promote genomic instability in DNA replication and modulate transcription and translation. These structures are found in promoter regions of many cancer-related genes such as c-MYC [1,2]. G4's have transient structural arrangements and can be unfolded by helicases, such as DHX36 [3]. The stabilization of G-quadruplexes by small organic molecules has shown promising results as an anticancer drug target [1]. Nonetheless, there are a great number of problems, such as high lipophilicity and lack of specificity towards specific G4s.

To overcome these obstacles, in this project, we propose to design, synthesize and evaluate multiple indoloisoquinoline derivatives as potential inhibitors of the interaction between c-MYC:G4 and its negative regulator, the helicase DHX36 [3]. The indoloisoquinoline scaffold was combined with a library of purchasable fragments to create a final database of compound derivatives. This dataset was then used in a computational Molecular Docking screening campaign targeting the recently resolved structure of c-MYC:G4 in complex with DHX36 [3], to identify the most promising inhibitors. Some of the compounds were synthesized and will be evaluated in the future, using *in vitro* assays, for binding and selectivity to the c-MYC:G4. The obtained results will be integrated into additional structure:function evaluations, and guide new computational predictions, synthesis, and functional validation.

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P18 | Validation of a Flow Apparatus for VLE measurements with water+ methanol binary mixture

Ferreira, Cristiana (1); Nobre, Luís C. S. (1,2); Nobre, Beatriz (1); Palavra, António M.F. (1); Nieto de Castro, Carlos (2) and Cristino, Ana F. (2)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

(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.

Vapor-liquid equilibrium (VLE) is one of the most important properties that can give an insight about the behavior of mixtures with temperature for separation purpose. VLE data have been actively measured and collected experimentally. However, due to the number of possible systems to study, it is realistically impossible to retrieve experimental data for all important systems. In addition, existing data sometimes have poor quality. [1] This lack of accuracy can in some cases be the reason for significant losses due to erroneous process design costing millions.

Accurate VLE data with alcohols is essential for processes that involves biofuels in order to compete with crude oil market and prevent global warming. Produced from renewable sources, these chemicals are pointed as alternatives for such market, due to their fuel properties and capability of increasing octane number, reducing exhaust gas emissions, enhancing combustion, or even replacing gasoline itself. [2]

This work aims to validate a flow apparatus to carry out accurate VLE measurements. According to the IUPAC recommendations, the mixture selected for the validation of the equipment was water + methanol, a well-known mixture with several sets of data in the literature for comparison [3]. Isothermal VLE measurements will be presented for this mixture from 373.15 K to 423.15K.

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P19 | Novel cathode material for metal-ion batteries - PEDOT:DS

Zeferino, Jorge F. (1); Santos, Daniel R. (1); Correia, Jorge P. (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

Poly-[3,4-ethylenedioxythiophene] (PEDOT) is an interesting electronically conducting polymer due to its ability of being doped with different polyanions [1], making it an easily tailored material. Being also a redox active material, its high reversibility of the redox process allows for their application in secondary batteries [2]. The most studied PEDOT:PSS is a polymer exhibiting pseudocationic redox behavior which, allied to its intrinsic flexibility, stimulated the investigation of their use as cathode in advanced alkali metal-ion batteries [3]. Dextran sulfate (DS) has been successfully used as a dopant for PEDOT [4] and the resulting polymer structure is shown in Figure 1.

In this work PEDOT:DS films were electrochemically synthesized over platinum under potentiostatic and galvanostatic control. The mass transfer events resulting from the redox transformations were assessed by combining the information retrieved from data taken by cyclic voltammetry (CV), probe beam deflection (PBD) and electrochemical quartz crystal microbalance (EQCM). Ex-situ ellipsometry measurements were also made to determine the optical parameters and the thickness of the electrosynthesized film. Characterization media consisted in acetonitrile solutions of $LiClO_4$ or $Mg(ClO_4)_2$. Further insights were taken from the PBD data by applying the mathematical tool of temporal convolution, which allows to quantify the ion participation in the redox conversion of the conductive polymer.

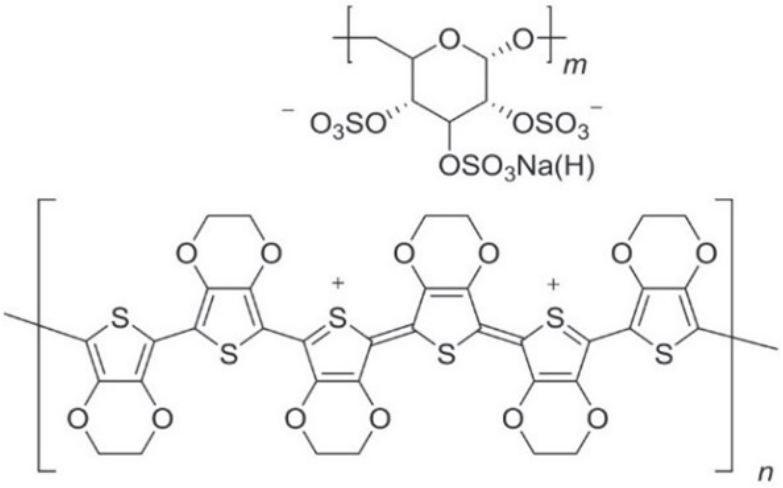

Figure 1: PEDOT:DS structure [4]

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P20 | The trihemic peroxidase from Escherichia coli proposed to receive electrons from the quinone pool

Oliveira, Ricardo (1)(2); Portela, Raquel (1)(2); Aguiar, Sara; Pauleta, Sofia R. (1)(2)

(1) Microbial Stress Lab, UCIBIO, NOVA School of Science and Technology, NOVA University Lisbon, Portugal.
(2) Associate Laboratory i4HB -Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Portugal

Pathogenic strains of *E. coli* have been shown to cause several human diseases. In some cases, the host's cells have different defence mechanisms, with some relying in the exposure of the pathogens to high concentrations of different radicals, like hydrogen peroxide.

Bacterial peroxidase composed of three-domains have been identified in the genome of pathogenic bacteria, but only a few were isolated and characterized to date [1]. These enzymes have an additional unique feature that is the presence of a N-terminal transmembrane helix, and are proposed to be involved in the detoxification of hydrogen peroxide in the periplasm [1] and to confer the ability to use H_2O_2 as a terminal electron acceptor in the absence of oxygen [2].

We have focused our studies on YhjA, a quinol peroxidase from *E.coli* [3]. This enzyme is proposed to receive electrons from the quinone pool, as it is composed by a C-terminal domain homologous to the classical dihemic bacterial peroxidases, binding two c-type hemes, an additional N-terminal domain, binding one c-type heme and a predicted transmembrane helix. The soluble domain of YhjA was already isolated and biochemically characterized, presenting quinol peroxidase activity *in vitro* (millimolar range KM values) using hydroquinone and menadiol (menaquinol analogue) as electron donors [3].

YhjA without the transmembrane domain, both wild-type and M125A variant - with a mutation on the proposed axial ligand of the N-terminal heme – were isolated and spectroscopically characterized, and their kinetic parameters using an artificial electron donor were determined and compared to validate the proposed axial ligand and infer the involvement of this heme in electron transfer required for catalysis. Inhibition studies were performed using known inhibitors of the peroxidase family. The isolation and characterization of the wild-type and M125A full-length YhjA are being carried out.

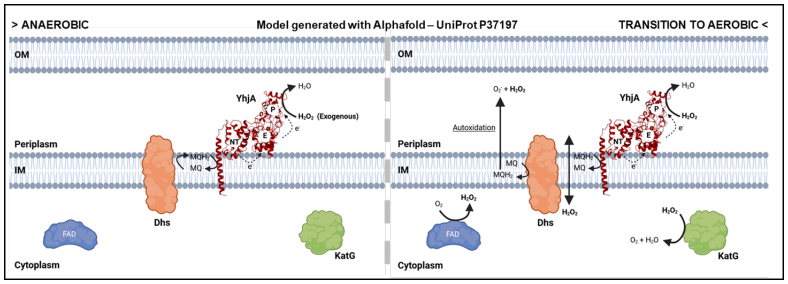

Figure 1: Proposed model for YhjA role in *E. coli* under anaerobic conditions and when transitioning from an anaerobic to an aerobic environment

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P21 | Synthesis of nucleoside analogs based on D-glucoronamide moieties as potential antibacterial agents

Moreira, Tânia (1); Manuel, Domingos (1) ; Neto, Euclydes (1); Nunes, Rafael(1) ; Frias, Maria (1) ; Filipe, Sérgio (1); Xavier, Nuno(1)

(1) CQE@FCUL

Synthetic nucleosides, nucleotides and their analogs or mimetics have occupied an important place in medicinal chemistry, with a number of compounds in clinical use to treat various types of cancers and viral infections [1]. These groups of molecules are prompted to interfere with nucleos(t)ide-dependent biological events that are crucial for the progress of various diseases [1]. The antimicrobial potential of synthetic and natural nucleos(t)ides has also been well documented [2]. Major issues associated with their clinical use include their low bioavailability and the emergence of chemotherapeutic resistance [1]. The design and synthesis of novel bioactive nucleoside/nucleotide-like structures that may overcome these limitations, potentiate alternative mechanisms of action and open new therapeutic opportunities is of significant interest. In this context, we report herein on the synthesis of a variety of nucleoside, nucleotide and sugar diphosphate analogs/mimetics constructed on D-glucuronamide templates, which are rather unusual glycosyl units in nucleoside chemistry, and comprising a 1,2,3-triazole moiety. The triazole unit was envisaged as a surrogate of a nucleobase or as a potential neutral and rather stable surrogate of a phosphate group when combined with other moieties such as phosphonate or amide to establish new potential neutral diphosphate group mimetics. The synthetic methodologies used azido pyranoses and D-glucofuranuronolactone as precursors and employed key steps such as azide-alkyne 1,3-dipolar cycloaddition, N-glycosylation, or Arbuzov reaction. Some compounds were subjected to antibacterial evaluation, from which one showed potent effect against the Gram-positive bacterial pathogen Streptococcus pneumoniae, with an activity higher than that of a standard antibiotic, thus tuning it a promising lead molecule for further investigations.

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P22 | Synthesis of new bisquinolizidine derivatives from bio renewable resources

Ferreira, Daniela R. (1); Durão, Raquel 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, DQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

Bisquinolizidine alkaloids, such as (+)-lupanine is found in several plants of the subfamily Faboideae including the genus Lupinus. This molecule is characterized by a common chiral bispidine core and possess a variety of biological activities, from antiarrhythmic and oxytocic properties to a partial agonist of the nicotinic acetylcholine receptor [1,2].

Our group have been developing methods for the sustainable isolation of these alkaloids [3]. Currently, our research interests include developing methodologies for the functionalization of bisquinolizidine alkaloids for medicinal chemistry applications. In this work, we present the syntheses of 17-substituted lupanine derivatives through the addition of Grignard reagents to the iminium ion formed from lupanine (Figure 1a) and N-substituted lupanine derivatives through alkylation reactions (Figure 1b).

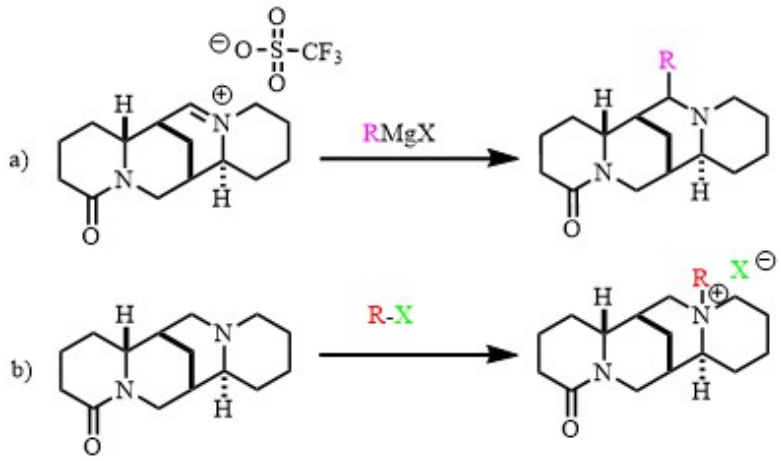

Figure 1: Reaction scheme of addition of Grignard reagents (a) and alkylation reaction (b).

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P23 | Sea urchin's adhesion - the relevance of glycoproteins

Ventura, Inês (1), Harman, Victoria (2), Beynon, Robert J. (2), Santos, Romana (1)

(1) Centro de Ciências do Mar e do Ambiente (MARE), Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
(2) Centre for Proteome Research, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK

Bioadhesion is vital for many aquatic organisms, impacting how these animals attach, move and feed in their habitats.[1] Currently, the best studied bioadhesives are from organisms that attach permanently (mussels, barnacles) or transitorily (limpets), while little is still known about reversible adhesion (sea urchins, sea stars). Our lab recently demonstrated the presence of high molecular weight putative adhesive/cohesive glycoproteins bearing N-acetylglucosamine residues in the form of chitobiose in *Paracentrotus lividus* adhesive disc epidermis and footprint.[2] Taking advantage of lectin affinity towards some glycans and glycan arrangement, we further investigated and characterized these *P. lividus* candidate adhesive/cohesive glycoproteins by performing lectin pulldowns. Followed by mass-spectrometry, the recovered glycoproteins were then identified, making use of the recently published transcriptome specific for *P. lividus* adhesive organs.[3] Within the identified glycoproteins, only those matching previously pinpointed adhesive candidates[3] were selected and detailly characterized with bioinformatic tools (*in silico* analysis).

Using this multidisciplinary approach, we pulled down high molecular weight glycoproteins containing GlcNac (simple and chitobiose) and GalNac (SBA), identifying for the first time five putative adhesive/cohesive sea urchins' proteins as glycosylated. Based on their biochemical characterization, we described (i) two large negatively charged proteins (Nectin and alpha-tectorin) and a smaller positively charged protein (uncharacterized protein), with a probable adhesive and/or cohesive function, (ii) an alpha-2-macroglobulin enzyme, possibly promoting adhesion/cohesion or having a protective role and (iii) a peroxidase, most likely involved in protein crosslinking, contributing to the cohesiveness of the adhesive. Providing a stronger and biocompatible alternative to commercial medical glues, the deeper characterization of these adhesive/cohesive glycoproteins represents a step forward towards the development of biomimetic sea urchin inspired bioadhesives.

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P24 | Isoniazid derivatives against drug-resistant tuberculosis do not perturb membrane raft domains

Terreiro, Joana F.P.R.(1); Marquês, Joaquim T.(1), de Faria, Catarina, F.(1), Santos, Susana(1), Martins, Filomena(1), de Almeida, Rodrigo F.M.(1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa.

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) which, despite being preventable, and mostly curable, is still one of the leading causes of mortality worldwide due to the growth of multi-drug resistant strains unsusceptible to currently available therapies. In this sense, a series of isoniazid derivatives already shown to be valid leads to overcome drug resistance in tuberculosis were synthesized [1, 2]. Four compounds, INH, INH-C10, N34 and N34red, were selected to study their interaction with different membrane model systems by steady-state and time-resolved fluorescence spectroscopy and the use of fluorescent membrane probes. Three biomembrane models were evaluated: two single-component systems, a gel and a fluid lipid bilayer, studied with the probes t-Pna and di-8-ANEPPS, and a ternary lipid bilayer containing sphingomyelin and cholesterol to mimic the mammalian plasma membrane, namely the presence of lipid rafts, studied by Förster's resonance energy transfer (FRET).

Studies with t-Pna revealed that all INH derivatives, especially INH-C10, caused a disturbance in the packing of the acyl chains in the lipid gel phase, with no effect on fluid membranes. The probe di-8-ANEPPS disclosed that all compounds studied disturbed membrane dipole potential, in both fluid and gel bilayers, suggesting an insertion at a superficial level of the biomembrane. Additionally, using FRET, it was possible to determine that none of the compounds influenced the size and organization of membrane lipid domains. All compounds incorporate into both gel and fluid lipid membranes.INH-C10, in particular, penetrates deeper into gel membranes suggesting that it might be able to incorporate into other dense membranes, such as the mycolic acids-rich Mtb cell wall, thus facilitating its delivery to the therapeutic target. FRET results indicate that both INH and INH derivatives can incorporate in cell-mimicking plasma membrane without disturbing its domain organization which is a good indicator of their therapeutic potential.

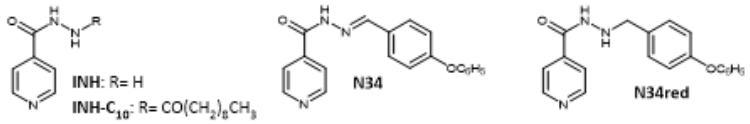

Figure 1:

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P25 | Crystallization and solubility studies of picolinic acid

Baptista, Diogo S.(1); Piedade, M. Fátima M.(1)(2); Esteves, Catarina V.(1);

(1) Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

(2)Centro de Química Estrutural, Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

Crystallization is a process used to obtain pure solids from solution. Indeed, it is an old and common method used everywhere. However, it remains unclear how molecules aggregate in solution and form a crystal. Many variables, for instance the temperature and the solvent used, can affect the crystallization outcome and the appearance of polymorphs (different crystalline structures of the same material). In order to synthetise only the desired solid, of a given compound, it is crucial to study the existence of polymorphism and what are the exact conditions in which we are able to obtain the pretended crystalline structure[1]. The study of a family of compounds with systematic variations in the molecular structure could help uncover the molecular mechanisms throughout crystallization. Therefore, picolinic acid, whose isomer, nicotinic acid and parent compounds, hydroxynicotinic acids, have been thoroughly studied in our group[2][3] and could constitute models for such study. This family of compounds has known biological relevance, namely picolinic acid, which is used to chelate several metals, in particular zinc and chromium and these specific chelates are sold as alimentary supplements.

In this work some results on both the solubility (obtained through the gravimetric method) and solid structure (by means of PXRD), in equilibrium with a saturated solution of picolinic acid, at different temperatures (293 - 323 K), in three polar solvents: water, ethanol (both protic solvents) and acetonitrile (aprotic solvent) will be presented. These results show us that picolinic acid is very soluble in water (for T $\approx$ 293 K, c(PA) $\approx$ 862.5 g.kg^{-1}), much less soluble in ethanol (c(PA) $\approx$ 57.1 g.kg^{-1}) and even less in acetonitrile (c(PA) $\approx$ 17.0 g.kg^{-1}). Regarding the structure in the solid-state, it does not have any significant changes between solvents and temperatures and its crystal system is monoclinic with a C2/c space group.

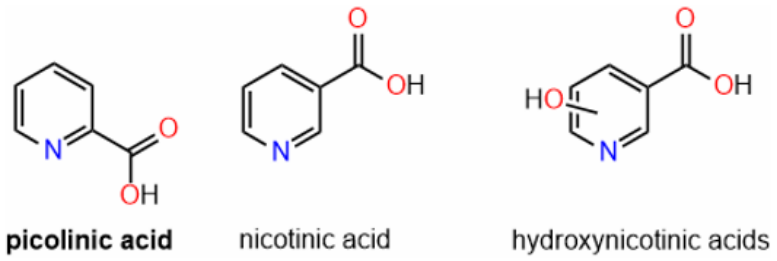

Figure 1: Molecular structures of picolinic, nicotinic and hydroxynicotinic acids

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This work was supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal (projects PTDC/QUI-OUT/28401/2017, LISBOA-01-0145-FEDER-028401, UIDB/00100/2020, UIDP/00100/2020 and LA/P/0056/2020).

P26 | Revealing the Chemical Universe of Fingermarks by FT-ICR-MS

Pereira, Mariana (1), Gomes, Nelson (2,3), Cordeiro, Carlos (1), Madureira-Carvalho, Áurea (2,3), Sousa Silva, Marta (1)

(1) Laboratório de FTICR e Espectrometria de Massa Estrutural, MARE-Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal;
(2) TOXRUN - Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal;
(3) REQUIMTE/LAQV, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira, no. 228, 4050-313 Porto, Portugal

Fingermarks are unique to each individual and commonly correspond to a complex pattern of ridges and valleys that are crucial in Forensic Sciences for human identification [1]. Besides the physical pattern, fingermarks contain a wide range of chemical constituents. Untargeted metabolomics based on extreme resolution, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), enables the detailed description of the chemical complexity of dermopapillary residues [2,3], leading to the identification of hundreds of chemical species and metabolites. Exogenous substances such as pharmaceuticals, drugs, personal care products, and food additives are of great forensic importance. However, the development of latent fingermarks generally requires the use of different reagents [4], making their characterization by mass spectrometry an extremely complex affair in a realistic scenario.

The purpose of this study was to obtain relevant chemical information from fingermarks in the presence of common fingermark developers like Instant White, Dragon's Blood, and Magnetic Latent Print Powder. The extracted metabolites were analysed by FT-ICR-MS on a 7 Tesla Solarix XR from Bruker, equipped with the ParaCell. All samples were analysed by direct infusion in positive electrospray ionization mode and processed in absorption mode. Metabolite identification was performed using the Human Metabolome Database (HMDB) - pharmaco and sweat metabolites. The results obtained allowed us to observe minimal differences in the identified chemical compounds between latent and developed fingermarks.

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P27 | Molecular characterization of a GntR transcription factor

C. Almeida, Beatriz (1); Kaczmarek, Jennifer (2); L. Jones Prather, Kristala (2); T. P. Carvalho, Alexandra (1)

(1) Center for Neuroscience and Cell Biology, Institute for Interdisciplinary Research, University of Coimbra, Portugal
(2) Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, United States

Synthetic biology circuits are promising tools for biotechnology applications. The GntR superfamily, known to regulate essential biological processes, gives an attractive opening in the development of controllable expression systems [1]. Several studies strongly suggest that the majority of FadR TFs (the largest family of the GntR superfamily) are metal-dependent [2]. Yet, the metal role is poorly understood in GntR TFs [2]. Previously, we have built a new homology model for the UxuR (a FadR TF)[3].

Our results show that Zn(II) coordination is essential for UxuR function since mutation to alanines prevents expression derepression by the inducer. Our findings provide a bright first understanding of the allosteric mechanism study of UxuR TF. Now we are searching for key residues involved in the underlying allosteric mechanism. Through Gaussian accelerated molecular dynamics and computational analyses, we are dissecting specific residues and interactions able to modify the aTF response to different ligands.

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P28 | Application of two modern microextraction approaches for *in vitro* and on-site assessment of VOCs emitted by *E. globulus* and *P. pinaster* leaves

C. Gonçalves, Oriana (1); R. Neng, Nuno(1); M. F. 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.

The incidence of heatwaves and periods of drought have been continuously increasing due to climate change. As this contributes to the development of intense forest fires, it is essential to develop new highly sensitive analysis methodologies that simplify the monitoring of volatile organic compounds (VOCs), to understand their influence in the extreme behaviour of forest fires [1,2]. In the last decades, novel sample preparation techniques, such as solid-phase microextraction (SPME) and bar adsorptive microextraction (BAμE), have been widely for trace analysis of diverse organic compounds, showing great performance in several applications due to the high selectivity of the different sorbent coatings used and simplification of the sample preparation step [3].

In this sense, the present work aimed the development of both an in-vitro SPME and on-site BAμE approaches using headspace (HS) sampling followed by gas chromatography coupled to mass spectrometry (GC-MS) analysis for monitoring five VOCs (α-pinene, β-pinene, myrcene, limonene and 1,8-cineole) emitted by the leaves of E. globulus and P. pinaster trees from Sintra (Portugal). The in-vitro assessment of the tree leaves by HS-SPME/GC-MS allowed the detection of concentrations of the case study VOCs, in the range between 9.2 $\pm$ 1.4 and 7828.0 $\pm$ 40.0 μg g^{-1}. Also, the maximum concentrations of these VOCs emitted by the tree crowns at 30 °C (0.01 - 1.26 $g.m^{-3}$) were under their lower flammability limits (38.2-$g.m^{-3} \leq$ LFL $\leq$ 38.8 $g.m^{-3}$). Furthermore, through on-site application of the HS-BAμE/GC-MS methodology it was possible to detect four VOCs (106 $\pm$ 23 to 311 $\pm$ 8 ng), showing to be a promising alternative for monitoring VOCs in forest environments, given its great simplicity, easy manipulation, and cost-effectiveness.

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P29 | Targeting pathogen cell wall biosynthesis to combat the menace of Tuberculosis

Delgado, T. (1,2) , Pais, J. P. (1); Estrada F. (1); Guedes, R. (1,3); Pires, D. (1); Anes, E. (1,3); Constantino, L. (1,3)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal;

(2) Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal;

(3) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Tuberculosis (TB) is one of the leading causes of death from an infectious disease [1] and the recent rise in multidrug resistant variants has worried the scientific community, creating an urgent need for the discovery of new drugs that are effective against both drug-susceptive and drug-resistant TB. A promising new target for such compounds is DprE1. This epimerase is essential for the synthesis of DPA, a vital precursor of arabinogalactan, one of the components of the mycobacterial cell wall. Numerous inhibitors of this target have been described, among them nitrobenzamides [2]. The literature available for nitrobenzamides potentially acting on DprE1 show a multitude of structural features, most of which can be classified in three parts: an aromatic amide containing a nitro group (NA), a linker and a terminal group (TG). It is believed that these inhibitors act on DprE1 through the formation of a covalent bond with Cys387. So far, the majority of the literature makes use of linkers that confer low flexibility to the structure [3].

Previous work in the group has led to derivatives that showed promising activities against Mtb. The results could be indicative of the importance of flexibility of these structures. As such, the present work makes use of the general DNB scaffold, but employing linkers that increase the flexibility of the structure, to capitalize on that property. To synthesise our library of compounds we started by joining the linker, by nucleophilic addition/elimination using an acid chloride, and then we joined the last aromatic moiety (TG) via Mitsunobu reaction. Compounds synthesized show great promise (MIC ranging from 30 to 150 nM), hence we are also developing a docking approach to try to confirm the mode of action of our inhibitors and to guide the synthesis of the next compounds.

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P30 | Regulatory T cell depletion unleashes an IFNγ^+ $\gamma\delta$ T cell response in the tumour microenvironment

V. Pinto-Santos, André(1) Lopes, Noella (1), Condeço, Carolina (1), Mensurado, Sofia (1) Silva-Santos, Bruno (1)

(1) Instituto de Medicina Molecular - JLA

$\gamma\delta$ T cells are potent anti-tumoral effectors, capable of orchestrating adaptive immune responses that control tumour progression. The anti-tumour potential of $\gamma\delta$ T cells is mostly attributed to their fast and strong production of IFNγ. However, the cellular and molecular regulators of IFNγ-producing $\gamma\delta$ T cells in the tumour microenvironment (TME) are largely unknown. Here, using a murine orthotopic model of breast cancer (E0771) implanted in a FOXP3-DTR transgenic mouse strain, we studied the impact of regulatory T (Treg) cell depletion on $\gamma\delta$ T cell responses.

We show that murine CD4$^+$ FOXP3$^+$ regulatory T cells exert an overt suppressive effect on IFNγ-producing $\gamma\delta$ T cells, as these were significantly increased in numbers upon Treg cell depletion, resulting in diminished tumour growth. Interestingly, the IL-17-producing $\gamma\delta$ T cell counterparts were not affected by Treg cell depletion. Strikingly, this depletion also led to changes in the myeloid compartment, eliciting a decrease in monocytes/ macrophages and an increase in neutrophils. We are currently addressing the impact of the myeloid changes on $\gamma\delta$ T cell responses, aiming at unravelling the mechanism of IFNγ-producing $\gamma\delta$ T cell accumulation upon Treg cell depletion. This work identifies a suppressive (direct or indirect) interaction between Treg cells and $\gamma\delta$ T cells in the tumour microenvironment. In the future, we expect our findings to contribute to the design of new therapeutic strategies to induce stronger anti-tumour responses.

P31 | Synthesis and structural characterization of new Ru-cyclopentadienyl complexes for potential application in triple-negative breast cancer therapy

Sá, Marco(1); Franco Machado, João(1,2); Garcia, M. Helena(1); Morais, Tânia S.(1)

(1) Centro de Química Estrutural, Institute of Molecular Sciences, DQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
(2) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10 (km 139, 7), 2695-066 Bobadela LRS, Portugal.

Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, representing 15%-20% of their cases, for which there is no clinical cure. Available treatments have limited efficacy and severe adverse effects, mostly due to their lack of selectivity towards tumoral cells.[1] Among them, cisplatin and its analogues are the only metallodrugs approved for cancer chemotherapy. However, despite well established in clinical practice, they also show innate or acquired drug resistance in addition to the toxic side effects. The urgent need of more efficient therapies has driven our research group into developing alternative metallodrugs that might overcome the stated limitations.

We have previously reported several ruthenium-cyclopentadienyl (RuCp) complexes that showed to be very effective *in vitro* and *in vivo* against several types of cancer, including breast cancer and TNBC.[2] Additionally, we have also reported the conjugation of one our most promising complexes ([RuCp(PPh_3)(2,2'-bipyridine)][CF_3SO_3], TM34) with peptides that target the fibroblast growth factor receptor (FGFR) that is often overexpressed by TNBC cells. These novel metallodrug delivery systems showed to be more selective towards $FGFR^+$ breast cancer cells than the free complex TM34.[3] Herein, we report the synthesis and characterization of new RuCp complexes intended to be used as the cytotoxic moiety of the stated metallodrug delivery systems for the potential therapy of TNBC. Two complexes of general formula [RuCp(PPh_3)(NN)][CF_3SO_3], where NN represents different bipyridine ligands monofunctionalized with an alcohol or carbonate ester group, were synthesized for the first time. Their structural characterization was achieved by NMR (1H, APT-13C, 31P and 2D COSY, HSQC, HMBC), FT-IR, UV-Vis, and Elementary Analyses. The stability of the complexes was also evaluated by UV-Vis in organic (100% DMSO) and aqueous cell culture media (5% DMSO/95% DMEM) over a period of 24 h.

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P32 | Purification of ssDNA scaffolds for DNA-origami nanostructures biomanufacturing

Silva-Santos, Ana Rita(1), Paulo, Pedro M. R. (2), Prazeres, Duarte Miguel F. (1)

(1) iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
(2) Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal

DNA-origami biomanufacturing relies on the use of asymmetric PCR to generate 500-3500 base pair (bp), object-specific, single-stranded DNA (ssDNA) scaffolds using the DNA of the M13 phage as template. Each scaffold is purified by agarose gel extraction, a technique that is laborious, limited, not scalable, presents low recovery yields and a low-quality product. We present two purification alternative strategies to purify ssDNA scaffolds from asymmetric PCR (aPCR) mixtures, which can be used in DNA-origami techniques. In both cases, aPCR was performed to generate 1000 nt-long single and double-stranded DNA (dsDNA) from M13mp18 genome. To isolate the target ssDNA from dsDNA and other PCR impurities, magnetic beads, anion-exchange (Q-ligand) and multimodal chromatography (CaptoTM adhere ImpRes) were explored. In the case of magnetic beads, carboxyl-modified magnetic particles were functionalized with a 20-nt oligonucleotide complementary to the 3' terminal of the 1000 nt-long ssDNA scaffold working as an affinity ligand towards the target ssDNA scaffold. Hybridization between the ssDNA scaffold in the aPCR mixture and the affinity beads was promoted at high LiCl concentrations. The dsDNA didn't hybridize and could thus be separated from the magnetic beads. Following washing, magnetic beads were heated up to denaturation temperatures and ssDNA were recovered in the solution by magnetic separation.

In chromatography, a stepwise gradient with increasing NaCl concentrations was used. Unused primers and oligonucleotides were washed-out in the flowthrough due to their low charge density. In anion exchange chromatography, the less-charged ssDNA was eluted before the dsDNA. In multimodal chromatography, however, the elution pattern was reversed, highlighting the importance played by hydrophobicity. Gel electrophoresis revealed that ssDNA-containing fractions are homogeneous and impurity free.

Finally, the recovered 1000-nt ssDNA was used to assemble 63-bp edge length tetrahedrons using site-specific short oligonucleotides (staples), thermal annealing and high magnesium concentrations. Agarose gel electrophoresis showed a high assembly yield and purity.

P33 | *In silico* study of the role of protein knots in mechanical stability and function

G. F. Ferreira, Sara; F. N. Faísca, Patrícia; Machuqueiro, Miguel
BioISI: Biosystems and Integrative Sciences Institute

Ubiquitin C-terminal hydrolases (UCHs) are papain-like cysteine proteases that hydrolyze the ubiquitin adduct, countering the ubiquitination process in proteins. This procedure consists of a posttranslational modification, being therefore involved in the regulation of both membrane trafficking and protein degradation pathways. Besides its importance in the ubiquitin-dependent proteolytic pathway, UCH-L1, one of the four UCHs present in the human genome, is also highly expressed in the brain. It is estimated to make up 1 to 5% of total neuronal protein [1], and has been characterized as being involved with Parkinson's, Alzheimer's, and other neurodegenerative diseases [2]. This protein has one of the most complicated 3D knotted structures yet discovered, where five crossings of the polypeptide backbone form a '5 2', or 'Gordian' knot.

UCH-L1 has been reported to unfold with three populated states, transitioning from its folded state to fully denatured via an intermediate stage where its α-helices are already unfolded, but the β-strands central hydrophobic core remains intact [3]. Therefore, the leading step is to understand how the presence of knots in these intermediates can influence the oligomerization process, and the overall stability of this protein. To achieve such a goal, we used MD simulations to study the conformational space of the knotted UCH-L1, and developed a computational protocol to access the mechanical stability of knotted conformations, including the native structure and selected intermediates. We will present the preliminary results of MD simulations with our truncated protein, both in the N- and C-terminal, in order to identify which residues are involved in the knotting and their role in the overall protein stability.

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P34 | Peptide dendrimers as transfection agents in DNA/RNA vaccines: an *in vivo* study

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

(1) BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

(2) Department of Chemistry, Biochemistry and Pharmacy, Bern University, 3012 Bern, Switzerland

In the past years peptide dendrimers with two or three amino acids in the branches have been reported to interact with biological molecules and cell membranes leading to good activity as antimicrobial agents, pathogenic biofilm inhibitors and superior vectors for DNA, siRNA and small oligonucleotides [1]. Recently, the use of such structures as vector molecules for mRNA and siRNA vaccines has been explored [2], which resulted in some promising peptidic dendrimers, namely MH13 and MH18. They are solely constituted by lysines and leucines, and contain two palmitoyl chains or a leucine tetrapeptide as hydrophobic cores, respectively. Furthermore, some mutations in MH18 from L- to D-amino acids, results in improved binding to siRNA, as well as improved resistance to proteolytic degradation [2]. However, these mutations also lead to inferior transfection efficiencies [2]. It is remarkable how little we know about the molecular mechanisms of their actions and the reasoning why some combinations lose or acquire specific properties [3].

In this work, we will present preliminary findings regarding the application of our state-of-the-art CpHMD methodology to the pH-dependent conformational space of MH18 and its variants composed of a different number (and position) of D-amino acids. These results are pivotal to help us choose the next steps of the project, where the interactions with lipid membranes and DNA/RNA are planned, to help experimentalists interpret their data, and to design new and improved peptidic dendrimers.

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P35 | Overview of the ongoing PhD thesis: "Assessment of risks, trends and mitigation strategies for the microplastics contamination in the marine environment"

Morgado, Vanessa (1,2), Palma, Carla (2), Bettencourt da Silva, Ricardo (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.
(2) Instituto Hidrográfico, R. Trinas 49, 1249-093 Lisboa

The concern with the contamination of the environment with microplastics is very trending nowadays due to the omnipresence of plastic materials. The awareness of this threat to the environment and human health has attracted the scientific community to the monitoring of microplastic contamination in several aquatic systems and matrices. The monitoring of the level and trends of the contamination by microplastics is essential to determine the relevance and potential sources of this contamination necessary to define strategies to reduce it. However, the impact of microplastics in aquatics systems is only understood if its contamination is characterized adequately and objectively.

This work aims to present an overview of the research developed during the first three years of the PhD thesis entitled "Assessment of risks, trends and mitigation strategies for the microplastic contamination in the marine environment"[1-3]. In the first and second years of this PhD, the research was mainly focused on the development of qualitative characterization techniques of the presence of microplastics in environmental samples. The qualitative chemical analysis was supported by automatic tools for spectra identification, in robust identification criteria to the variability of the spectra, and in the assessment of criteria vulnerability to misidentifications. In the third year, the research was mainly focused on the development of quantitative characterization techniques. The quantitative chemical analysis was supported by the development of, as far as we know, the first methodology for validation and uncertainty evaluation of microplastic contamination determination. Reporting results with uncertainty ensures the objectivity and quality of the determination of environmental contamination. This work represents a relevant contribution to improving the quality of the analyses of microplastics in environmental samples and to the achievable harmonization of analytical protocols.

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P36 | Synthesis of new carbohydrate based anion receptors

Daniel, Sofia (1); Cachatra, Vasco (1); Moiteiro, Cristina(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

Anion recognition represents a key role in several important biological processes, such as homeostasis maintenance through protein ion channels. Channelopathies are a series of serious pathologies associated these channels and their deregulation is a key factor in several diseases, including cystic fibrosis (CF). CF is a genetically inherited disease caused by a defective transmembrane transport of chloride anions. Therapeutic options rely on treatment of the symptoms, while there is still no cure.[1]

Our group has been focused on developing new compounds that can interact with different anions to improve the regulation of the ion channels, with promising results in a family of bis-urea with a central 2-aminobenzylamine moiety linked to either a thiophene or a benzo[b]-thiophene ring.[2] In this work, we present new bis-urea based compounds bearing carbohydrate moieties to further access their role in improving binding affinity towards several biological important anions, such as chloride and carboxylates.

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P37 | Functional characterization of putative miRNA-like sequences encoded in the HIV genome

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

(1) BioISI - Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa
(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 (Al4Animals), Avenida da Universidade Técnica, 1300-477 Lisboa
(4) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa

The Human Immunodeficiency Virus (HIV) is an RNA virus belonging to the genera of lentiviruses. Recent strategies to manage HIV infection focus on miRNAs as therapeutic targets. These are small non-coding RNAs (sncRNAs) with 19-24 nucleotides long, involved in gene expression regulation. An increasing number of miRNAs encoded by the host genome are being identified with an impact on HIV infection. In contrast, viral miRNAs are not as well studied. It is generally assumed that RNA viruses can't encode miRNAs, based on the notion that presence of canonical miRNA genes would result in a useless cleavage of the viral genome and transcripts.

Previous work from the lab [1] used high-throughput sequencing to profile the expression of sncRNAs in *in vitro* stimulated human naïve CD4 T cells in response to HIV infection. Analysis of sequencing reads that map exclusively to the viral genome revealed the existence of putative miRNA-like molecules encoded by HIV-1 and HIV-2 (Amaral et al, in preparation). In this work, we aim to experimentally validate these sequences as bona-fide miRNA genes by determining if they comply with the fundamental characteristics of canonical miRNA biogenesis: Dicer-dependent processing of a precursor hairpin and association with Argonaute proteins to negatively regulate targets.

Candidate pre-miRNA hairpin sequences were cloned in an expression vector and assayed in transfected HEK cells. With the intention of testing the dependence on secondary structure formation, mutants that prevent hairpin formation were generated and assayed. Preliminary results using the stem-Loop RT-qPCR method to detect mature miRNAs reveal a difference between mutant and wild-type plasmids, suggesting that the formation of these transcripts depends on a hairpin-like secondary structure. We are currently confirming these results through hybridization-based methods, as well as testing for Dicer dependency to obtain conclusive evidence for the existence of miRNA-like molecules encoded in the HIV genome.

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P38 | Novel pH-responsive ruthenium-peptide conjugates for the precision therapy of metastatic breast cancer

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

(1) Centro de Química Estrutural, Institute of Molecular Sciences, DQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
(2) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10 (km 139, 7), 2695-066 Bobadela LRS, Portugal.
(3) BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

Breast cancer is the most common and lethal tumour in women. It's treatable if early diagnosed, but incurable when advanced or metastatic. Indeed, patients with metastatic breast cancer (MBC) have a median survival time of 2 years, because there is no specific nor suitable treatment, capable of reaching cancer metastasis. Instead, patients are treated with less efficient drugs that show severe adverse effects due to lack of selectivity for tumoral over healthy tissues [1]. Thus, there is a global need for alternative therapeutic approaches.

Aiming a precision therapy for MBC that overcomes the chemotherapeutics in current use, we developed novel pH-responsive ruthenium-peptide conjugates (RuPC) that can selectively target and be activated by MBC cells to promote enhanced therapeutic efficacy and reduced secondary effects. The RuPC contain a peptide with high affinity/selectivity for the fibroblast growth factor receptor (FGFR) overexpressed by MBC cells, conjugated to a cytotoxic ruthenium complex through a linker sensitive to the acidic tumoral microenvironment. The latter allows site- and time-specific release of the active species into cancer cells, sparing the healthy tissues (Figure 1). The most suitable positions for peptide conjugation were determined by molecular dynamic simulations [2]. Herein, we report the synthesis, characterization, and biological evaluation of new pH-responsive RuPC, where the peptide is conjugated to the complex through different positions. The conjugates were obtained by an innovative ultrasound-assisted solid-phase approach. The drug release profile was determined in solution at pH values that mimic the tumour microenvironment and the bloodstream. The *in vitro* cytotoxicity was evaluated in a panel of human breast cancer cells with different levels of FGFR expression. The new RuPC showed controlled release of the Ru complex in its active form and selective antiproliferative activity against FGFR(+) breast cancer cells, suggesting their potential use as novel agents for the precision therapy of MBC.

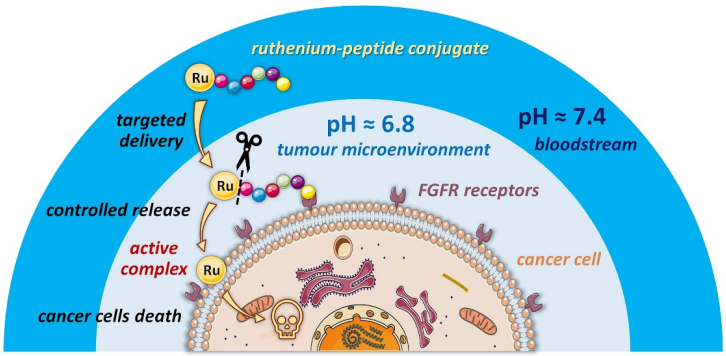

Figure 1: Proposed mechanism of action of the pH-responsive ruthenium-peptide conjugates.

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P39 | HIV-Based Virus-Like Particles: The Next Step in Targeted Therapy

Martins, Sofia A. (1), Cabo Verde, Sandra (1,2), Correia, João D.G. (1,2), Melo, Rita (1)

(1) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139,7; 2695-066 Bobadela LRS, Portugal;
(2) Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139,7; 2695-066 Bobadela LRS, Portugal

Introduction: Virus-like particles (VLPs) are virus-derived nanoplatforms composed of one or more proteins with the capability to self-assemble that lack viral genetic material [1]. VLPs arise as promising nanoparticles (NPs) that can be exploited as vaccines, drug delivery vehicles and carriers of imaging agents [1]. Antibody constructs, such as single-chain variable fragments (scFv), have been relevant tools to direct NPs to their target [2]. A vector containing the scFv of an antibody against the human epidermal growth factor receptor 2 (HER2) fused to the human immunodeficiency virus (HIV) protein gp41 was previously constructed [3]. The aim of this work was to produce and characterize HIV-1-based VLPs directed at HER2, with the overarching goal of establishing the proof-of-concept of this approach for targeted therapy.

Materials and Methods: The HIV-1-based VLPs were produced through transient transfection of HEK-293T cells. Enzyme-Linked ImmunoSorbent Assays (ELISA) and Western Blot assays were performed to validate the produced VLPs and to confirm the presence of the scFv at the surface. Transmission electron microscopy (TEM) was performed to characterize the constructed VLPs and the water-soluble tetrazolium salt (WST-1)-based assay was conducted to assess the cytotoxicity of the constructed VLPs in HER2-positive (SK-BR-3) and HER2-negative (MDA-MB-231) breast cancer cells.

Results: The VLPs were obtained with concentrations that range from 100 to 1000 pg/ml, and the presence of the fragment of interest was confirmed. TEM analysis showed that the VLPs assembled correctly and display a diameter between 100 and 200 nm. Preliminary proliferation studies show that the VLPs are not cytotoxic to SK-BR-3 and MDA-MB-231 breast cancer cells.

Conclusions: HER2-specific HIV-1-based VLPs were successfully assembled. The preliminary obtained results prompt further studies to establish these nanostructures as targeted delivery platforms.

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P40 | Direct electrochemical oxidation of abietanes

Martins, Inês S. (1); Coelho, Jaime A. S. (2); Afonso, Carlos A. M. (1)

(1) Research Institute for Medicines - Faculty of Pharmacy, University of Lisbon;
(2) Centro de Química Estrutural - Institute of Molecular Sciences, Faculty of Sciences, University of Lisbon

Colophony, a natural resin obtained from coniferous trees through distillation of gum, is constituted by a group of diterpenes known as abietanes, which, along with its derivatives, has been found to have a wide variety of interesting biological activities, including the antimicrobial, antiviral, antitumoral, anti-inflammatory, antiulcer and gastroprotective activities. Constituents of this resin have a wide range of industrial applications, including synthetic rubbers, adhesives, paints, printing inks and fragrances. [1,2] The benzylic oxidation of dehydroabietic acid, an abietane from colophony, and its methyl ester derivative, has been reported using oxidative protocols, such as using Jones reagent, Swern oxidation or either using Chromium trioxide in stoichiometric or catalytic quantities (Figure 1). However, these protocols fail in the context of sustainability for several reasons, such as the use of toxic reagents and stoichiometric amounts.

Herein, we report an electrochemical method for the benzylic oxidation of dehydroabietic acid, an alternative greener protocol for the formation of the benzylic ketone in very good yields using modern electrochemical methods (Figure 1). Moreover, this method can be applied to the corresponding methyl ester derivative. [3]

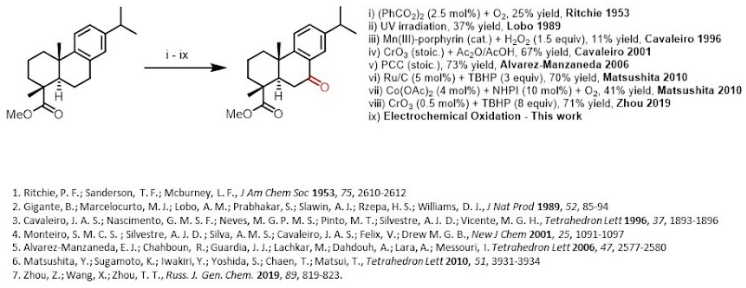

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Figure 1: Electrochemical oxidation of dehydroabietic acid (DHA) and its methyl ester derivative (MDHA).

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P41 | Prediction of tissue specific cancer driver genes prediction through network neighbors

Lopes, Paulo (1); Coelho, Alexandre (2); Pinto, Francisco (2)

(1) Departamento Química e Bioquímica ;
(2) BioIsi

Cancer driver genes are usually described as genes that are frequently mutated in cancer samples. These genes tend to be associated with a few different cancer cases, however, some drivers can be involved in many cancer types [1]. We recently observed that genes known to be cancer drivers in 6 or more tissues have significantly higher expression values in those tissues when compared to the remaining ones. Protein-protein interaction (PPI) networks play an important role in biological processes and diseases, with the disease phenotype being linked to the genes surrounding the causal gene [2].

Here, we propose to develop a machine learning-based pipeline able to predict cancer driver genes tissues. This pipeline uses expression values of the known driver gene and its neighbors to make the prediction, together with feature selection steps that will serve as a filter to find the best predictors. Results will hopefully provide a more specific method for the prediction of cancer driver genes and their tissues.

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P42 | Investigating the role of IL-17 $\gamma\delta$ T cells in peripheral nerve regeneration

Ghilas, V.* (1,2); Darrigues, J. (2); Almeida, V. (2,3); Saúde, L. (2); Silva-Santos, B. (1,2); Bombeiro, A.! (2); Ribot, J.! (2)

[1] FMUL – Faculdade de Medicina, Universidade de Lisboa, Portugal
[2] iMMJLA – Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal
[3] NMS – NOVA Medical School, Universidade NOVA de Lisboa, Portugal
*Corresponding author: vghilas@medicina.ulisboa.pt !Both contributed equality to the supervision

IL-17 producing $\gamma\delta$ T-cells ($\gamma\delta$17 T-cells) are a multi-faceted population as they not only play roles in cancer, autoimmunity and host defense, but also contribute to tissue homeostasis maintenance1. $\gamma\delta$17 T-cells regulate short-term working memory in the central nervous system (CNS)1, promote muscle regeneration2 and tissue sympathetic innervation1. The peripheral nervous system, contrary to the CNS, can regenerate through a process of major immunological involvement3. Given the aforementioned roles of $\gamma\delta$17 T-cells we hypothesize that these cells play a major role in peripheral nerve regeneration. C57BL/6 male mice were submitted to the unilateral sciatic nerve crush injury. Nerves and popliteal lymph nodes were dissected out at different time points for the characterization of the IL-17 producing cells landscape by flow cytometry. Motor function of Il17a+/+ and Il17a−/− mice was evaluated through sciatic function index obtained by walking track test. Regeneration hallmarks expression levels were evaluated by fluorescence microscopy. Our data shows an accumulation of $\gamma\delta$17 T-cells in the sciatic nerve and popliteal lymph nodes 3-5 days after injury. Of note, $\gamma\delta$17 T-cells were responsible for over 50% of total IL-17 in the crushed nerve. Importantly, Il17a−/− mice were unable to fully recover motor function contrary to Il17a+/+ littermate controls. At the histological level, Neurofilament and Myelin Protein Zero (MPZ) expression levels were not affected by IL-17 at any timepoints, whereas Growth Associated Protein 43 presented a 2-fold increase 7 days post-injury in the Il17a−/− group.

Our preliminary results indicate that axonal growth is seemingly not affected by the lack of IL-17. Therefore, the deficient motor function recovery observed in Il17a−/− mice may result from impaired nerve impulse transmission, possibly caused by aberrations in myelin structure or thickness myelination. Altogether, our findings suggest a critical role for $\gamma\delta$17 T cells in nerve regeneration, in which IL-17 acts towards motor functional recovery.

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P43 | Spectral feature occurrence as a viable alternative to intensity-based data for profiling and discrimination in untargeted metabolomics

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

(1) Laboratório de FTICR e Espectrometria de Massa Estrutural, MARE-Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Untargeted metabolomics experiments with high-resolution instruments yield numerical matrices with signal intensities of detected metabolites, which can be highly variable due to high metabolome sensitivity. To minimize this effect, sequential data pre-treatments are applied to extract biological information for data characterization and interpretation. However, the sets of metabolites detected are characteristic of biological systems, while being less affected by variability in comparison with intensity data. Thus, we consider our novel methodologies, based on the rarely used spectral feature occurrence for metabolic profiling and class discrimination, to be competitive with the traditional intensity-based workflow, while providing complementary information by extracting relevant data from feature importance metrics.

To this end, we developed two new approaches based on spectral feature occurrence, Binary Simplification (BinSim) [1], and using graph properties of Mass-Difference Networks built for each sample of a dataset (sMDiNs) [2]. BinSim applies binarization to feature occurrence, while sMDiNs build networks where features are nodes linked by specific mass differences representing chemical transformations. Then, on diverse benchmark datasets, we empirically compared the performance of supervised and unsupervised statistical methods in discriminating samples either using these approaches or using combinations of data pre-treatments on the intensity matrices. Both BinSim and the Degree profile graph property of sMDiNs led consistently to highly similar performances to the best pre-treated intensity data. Additionally, unique information was extracted using these approaches, such as BinSim highlighting important biomarker-like features and sMDiNs allowing to rank chemical transformations importance for class discrimination, highlighting the tools' potential. Thus, methodologies using spectral feature occurrence are viable alternatives to the usual intensity-based workflow, allowing the highlighting of usually overshadowed data and providing complementary information to give a fuller picture of the biological systems in question.

[1] Traquete, F. et al. (2021) Metabolites, 11.
[2] Traquete, F. et al. (2022) Front. Mol. Biosci., (Submitted)

We acknowledge support from Fundação para a Ciência e a Tecnologia (Portugal) through projects PTDC/BAA-MOL/28675/2017, UIDB/04292/2020, Investigator FCT program CEECIND/02246/2017 to M.S.S., and 2021.06370.BD to F.T., from the Portuguese Mass Spectrometry Network (LISBOA-01-0145-FEDER-022125) and the Europe and Union's Horizon 2020 research and innovation programme (grant agreement 731077) funded Project EU_FT-ICR_MS.

P44 | PyBindE: Developing an In Silico Tool to Study and Quantify Protein Binding Affinities

Vitorino, João (1); Reis, Pedro (1); Machuqueiro, Miguel (1)

(1) BioISI – Biosystems & Integrative Sciences Institute, Department of Chemistry and Biochemistry

Given the high importance of binding interactions between biomolecules in living organisms, this is a topic that naturally results in one of the most densely populated fields of scientific research. In order to uncover the underlying forces that factor into binding affinities, it is essential to study energetics in molecule interactions. To that end, computational approaches such as Molecular Dynamics simulations can provide us with conformational details at atomic-level. One might use several approaches to calculate binding free energies, with single-trajectory MM-PBSA being a particularly useful one, in cases where the relative energy differences between configurations are concerned. We have recently developed a new implementation of such an approach — PyBindE [1] — which was used to study the binding and aggregation potential of β2-Microglobulin, exposing the most significant forces that contribute to the binding of each dimer, ranking the stability of each binding mode, and predicting their oligomerization profiles [2].

PyBindE is being further expanded to allow the study of the role of amino acid protonation states in complex stability, and the study of systems composed of membrane proteins and their interactors, such as in the case of the (de)activation of GPCRs and their interaction with known drugs.

Here, we shall provide a simple overview of the theory and approximations of our implementation, as well as the results of published and on-going projects, and how it can be used to tackle more complex systems.

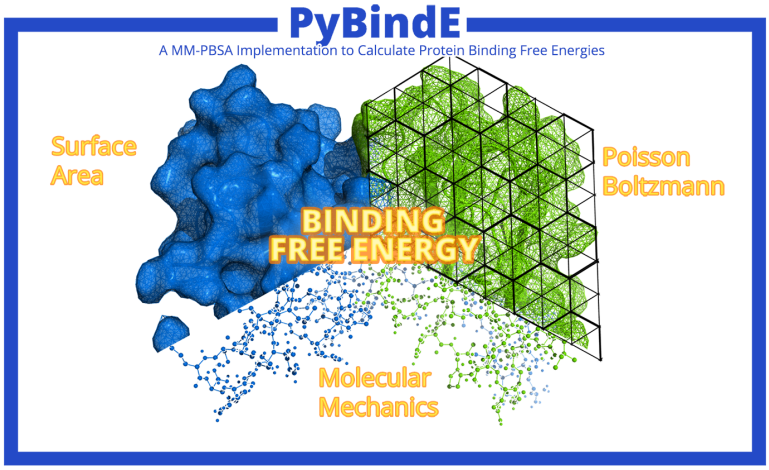

Figure 1: PyBindE - An MMPBSA implementation to Calculate Protein Binding Energies

[1] Vitorino, JNM; PyBindE: Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculations in protein-protein and protein-ligand systems. Github; Available: https://github.com/mms-fcul/PyBindE

[2] Oliveira NFB, Rodrigues FEP, Vitorino JNM, Loureiro RJS, Faísca PFN, Machuqueiro M. (2021) Comput Struct Biotechnol J.;19: 5160–5169.

The authors acknowledge financial support from Fundação para a Ciência e Tecnologia, Portugal, through grant CEECIND/02300/2017 and projects PTDC/FIS-OUT/28210/2017, UIDB/04046/2020, and UIDP/04046/2020.

P45 | Role of CFTR in Airway Epithelial Polarization and Differentiation

Rodrigues, Cláudia S. (1); Pankonien, Ines (1); Ramalho, Sofia S. (1); Farinha, Carlos M (1); Amaral, Margarida D. (1)

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

Background: Cystic Fibrosis (CF), the most common life-threatening monogenic disorder among Caucasians, is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, encoding a CL^- and HCO_3^- channel protein expressed at the apical plasma membrane (PM) of epithelial cells [1,2]. The most common CF-causing mutation, F508del, leads to defective CFTR PM traffic [3]. Other CFTR mutations cause decreased protein production, defective channel gating/conductance (e.g., G551D), decreased PM stability or total absence of CFTR protein [1]. CFTR has been associated to other cellular processes than its major function as an anion channel which include epithelial polarization, differentiation, regeneration but also cancer [4]. However, the mechanisms by which CFTR regulates epithelial polarization and differentiation are poorly understood.

Objective: The main objective is to understand how dysfunctional CFTR triggers impaired airway epithelial differentiation in CF.

Methods: Cystic Fibrosis Bronchial Epithelial cells expressing *wt*-, F508del-, and G551D-CFTR will be grown on permeable supports and the expression of epithelial markers will be assessed by Western blot (WB) and immunofluorescence (IF). In addition, a multipotent basal cell model of airway epithelial differentiation (BCi-NS1.1), previously shown to be able to differentiate into the multiple airway epithelial cell types [5] will be used. CRISPR/Cas9 gene editing will be applied to introduce G551D mutation into the CFTR gene. The differentiation into the various cell types over a 30-day period at Air-Liquid Interface culture will be assessed and compared to *wt*- and F508del-CFTR (previously generated in the Amaral lab) cell lines by WB and IF. Ussing chamber experiments will be performed to analyse the CFTR function. Results: CRISPR/Cas9 for G551D-CFTR has been successfully performed on BCi-NS1.1 cells and single cell clones are currently being screened/analysed. Further studies are underway to characterize the different cell models and the respective CFTR mutations regarding their impact on airway epithelial polarization and differentiation.

[1] De Boeck, K, Amaral, MD. (2016) Progress in therapies for cystic fibrosis. The Lancet Respiratory Medicine [PMID:27053340].

[2] Riordan, JR, Rommens, JM, Kerem, B et al. (1989) Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. Science (80-.). [PMID:2475911].

[3] Amaral, MD. (2004) CFTR and chaperones: Processing and degradation. Journal of Molecular Neuroscience [PMID:15126691].

[4] Amaral, MD, Quaresma, MC, Pankonien, I. (2020) What role does cftr play in development, differentiation, regeneration and cancer? International Journal of Molecular Sciences [PMID:32365523].

[5] Walters, MS, Gomi, K, Ashbridge, B et al. (2013) Generation of a human airway epithelium derived basal cell line with multipotent differentiation capacity. Respir. Res. [PMID:24298994].

Work supported by UIDB/MULTI/04046/201304046/2020 and UIDP/04046/2020 centre grants (to BioISI). CR is recipient of PhD fellowship from FCT/MCTES (Portugal).

P46 | Refractive index measurements for the system 1-pentanol + [C2mim][EtSO4], with interest to absorption refrigeration processes

Miranda, David(1); Nobre, Luis C.S. (1,2); Cristino, Ana F. (1); Santos, Fernando(1); Nieto de Castro, Carlos A. (1),Santos, Ângela F.S.(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.
(2) Centro de Química Estrutural, Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

Absorption refrigeration processes, due to their use of renewable energy, have come to be increasingly favored over the standard compression cycles. Absorption refrigeration systems, however, do often entail the use of binary mixtures (absorbent + refrigerant) that are either toxic or corrosive. In an effort to counter these environmental hazards, and owing to their considerable stability and environmentally-friendly nature, ionic liquids have been considered as potential absorbents [1]. Imidazolium-based ionic liquids have also been studied as alternatives.

The aim of this work was to provide data of refractive index of binary mixtures of 1-ethyl-3-methylimidazolium ethyl sulfate, [C_2mim][$EtSO_4$], and 1-pentanol, covering the entire range of composition and for temperatures T = (283.15 to 343.15) K and P = 0.1 MPa. With such data, we may infer the behavior, at the molecular level, by combining it with other thermophysical properties, like density. Also interesting is the comparison between our system, ([C_2mim] [$EtSO_4$] + 1-pentanol), and those described in the literature that use the same IL but other alcohols, ([C_2mim] [$EtSO_4$] + alcohol.)

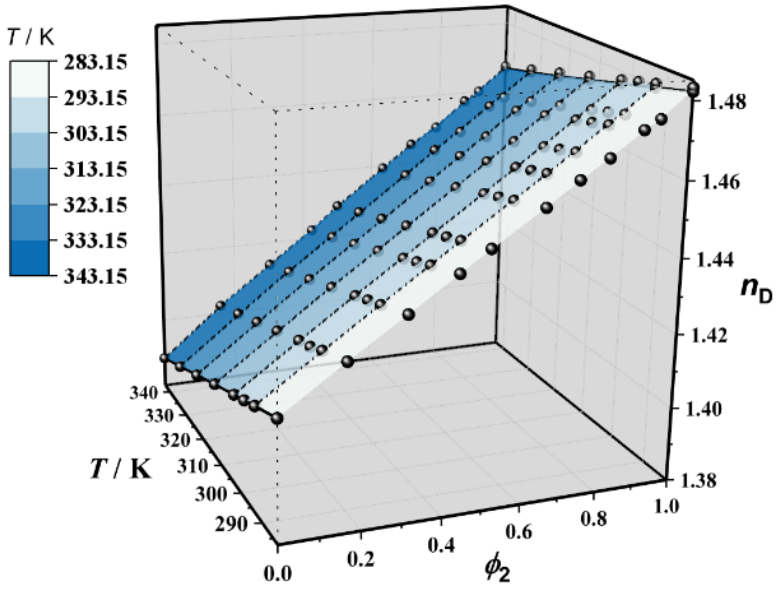

Figure 1: Experimental results on the refractive index of 1-pentanol + [C_2mim][$EtSO_4$] as a function of the volume fraction in the range of T = (283,15 to 343,15) K and P = 0,1 MPa.

[1] Moreno, D., Ferro, V. R., de Riva, J., Santiago, R., Moya, C., Larriba, M., & Palomar, J. (2018). Absorption refrigeration cycles based on ionic liquids: Refrigerant/absorbent selection.

Centro de Química Estrutural is a Research Unit funded by Fundação para a Ciência e Tecnologia (FCT) through projects UIDB/00100/2020 and UIDP/00100/2020. Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020. ILGerants is a project funded by FCT through project LISBOA-01-0145-FEDER-032066 + PTDC/EQU-EQU/32066/2017.

P47 | Coarse-Grain Parameterization of Ceramide for the Martini 3 Forcefield

Sá Vieira, Gonçalo(1), Melo, Manuel N.(1)

(1) Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

Ceramides (CERs) are a class of lipids among the least polar ones that has been found in different locations such as the Stratum Corneum, the skin's outermost layer, and the mitochondrial outer membrane. Within the latter, CERs have been linked to the formation of large protein permeable pores, which allow the release of proapoptotic proteins, upon regulated cell death. Since the release of the new Martini 3 Forcefield, no parameters have been published for CERs, despite its wide relevance. To fulfill this gap, we are developing new parameters for CERs headgroup to be used in Coarse-Grain (CG) simulations with this updated forcefield.

Fully atomistic simulations of either CER headgroup alone in water, or the whole C16-CER in a bilayer of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), were employed to obtain reference data. Headgroup beads were assigned according to the rules of Martini 3, while ensuring the same hydrophobic/hydrophilic character and solvent accessible surface area (SASA) of the reference data. Tails' beads were assigned alike DPPC ones, which have already been validated, due to its similarities. CG mappings of the atomistic simulations were furtherly performed to develop topology parameters so that CG distances, angles, and dihedrals of the headgroup matched those from reference data. Measurements of the area per lipid were also applied to furtherly validate the parameters. Our preliminary results show that the developed parameters greatly resemble the atomistic behavior, which, when fully validated, will constitute a remarkable tool for a plethora of membranal CG simulations.

FCT (Fundação para a Ciência e Tecnologia), ITQB (MOSTMICRO).

P48 | Overcoming multidrug resistance in lung cancer: a preeminent role for Ru cyclopentadienyl agents

Teixeira, Ricardo G. (1), Belisario, Dimas (2), Riganti, Chiara (2), Saponara, Simona (3), Stefanova, Denitsa (4), Tzankova, Virginia (4), Yordanov, Yordan (4), Garcia, Maria Helena (1), Tomaz, Ana Isabel (1), Valente, Andreia (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.
(2) Department of Oncology, University of Torino, Torino, Italy.
(3) University of Siena, Department of Life Sciences, via Aldo Moro, 2, 53100, Siena, Italy.
(4) Medical University of Sofia, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, 2 Dunav Str., 1000, Sofia, Bulgaria.

Multidrug resistance (MDR) is set as responsible for 90% of treatment failure in many forms of cancer therapy. MDR involving the action of cell efflux pumps is one of the main mechanisms by which many cancers develop resistance to traditional chemotherapy drugs. As a solution to overcome this limitation, we developed a new strategy to increase the effectiveness of cisplatin (cisPt) in non-small cell lung cancers (NSCLC)[1], where this drug is used as the first-line treatment, by targeting the inhibition of cell efflux pumps. Our approach is based on organometallic Ru-cyclopentadienyl ("RuCp") compounds of general formula $[Ru(\eta^5\text{-}C_5H_4R)(bipy)(PPh_3)][CF_3SO_3]$ (where R = CHO, CH_2OH or CH_2Biotin, and bipy = 2,2' bipyridine functionalized ligands) for which the results of a recent structure-activity study highlighted their activity against four types of NSCLC cells with different expression levels of P-gp and MRP1 transporters (namely A549, NCI-H228, Calu-3 and NCI-H1975).

Within the six more promising compounds, we found that, when administered at non-cytotoxic doses, compounds containing the 4,4'-dimethyl-2,2'-bipyridine ligand (RT150 and RT151) were able to significantly increase cisPt activity against resistant cells by targeting P-gp and MRP-1 transporters, thus acting as preeminent collateral sensitizers (CS). We investigated the effects of RT150 and RT151 on cell migration using the scratch healing assay demonstrating that both compounds significantly inhibited the migration of endothelial EA.hy926 cells, without increasing the number of dead cells. Altogether, the results gathered show that RT151 in particular holds great potential as a CS promoting candidate for novel therapeutic strategies in lung resistant cancers. Further preclinical *in vivo* studies in mice are currently under evaluation to confirm the *in vitro* findings.

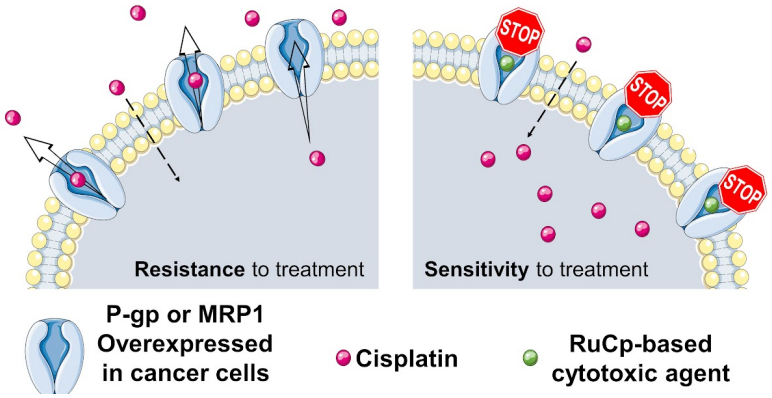

Figure 1: "RuCp" compounds increase cisplatin activity by inhibiting efflux pumps.

[1] Teixeira, R.G. et al. (2021), Inorg. Chem. Front., 8, 1983-1996.

This work was funded by Fundação para a Ciência e Tecnologia (FCT), I.P./MCTES through national funds (PIDDAC) – UIDB/00100/2020 and PTDC/QUI-QIN/28662/2017. R.G.T. thanks FCT for his Ph.D. Grant (SFRH/BD/135830/2018) and A.V. acknowledges the CEEDCIND 2017 Initiative (CEEDCIND/01974/2017). D.S., V.T. and Y.Y. are grateful to National Science Fund of Bulgaria (KP-06-COST/1/18.8.2021). All authors acknowledge COST Action 17104 STRATA-GEM.

P49 | The analysis of *Staphylococcus aureus*' NDH2 CoarseGrain simulations might be the beginning to unveil a future therapeutical target

Barriga, Rodrigo (1); M. Pereira, Manuela (2); N. Melo, Manuel (1)

(1) Instituto de Tecnologia Química e Biológica António Xavier, Lisbon, Portugal;
(2) BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences of University of Lisbon, Lisbon, Portugal

Some strains of *Staphylococcus aureus* have been gaining resistance to several different antibiotics, raising the possibility of a future pandemic. Therefore, ways of diminishing their virulence are of the utmost importance. Studies have shown a connection between Type II NADH: menaquinone oxidoreductase (NDH-2) and *S.aureus*' virulence. NDH-2 has thus promising therapeutical potential, highlighting the importance of studies of its role in respiration in general and of its binding site mechanisms in particular.

In this work, we have employed Coarse-Grain (CG) simulations to represent in silico the substrate approximation that occurs in the binding site in vivo. All the compounds related to this study, such as FAD (NDH-2 prosthetic group), NADH/NAD+, menaquinone/menaquinol and derivatives, were all parameterised using the most recent Martini 3 force field parameters. To perform the CG simulations, an *S. aureus* membrane of representative lipid composition was modelled together with NDH-2. Preliminary results show the preference of quinones for one of the NDH-2 binding sites (there is a separate site for NADH that the quinones do not visit). In addition, the reduction of quinones to quinols lowers their affinity for the binding site, thus promoting product egress. This hints that the binding site environment may be specifically tuned to discriminate the quinone from the quinol as a means to increase turnover. Our promising results show that CG Molecular Dynamics (MD) is well suited to studying NDH-2 substrate/product dynamics and could possibly be extended to other *S. aureus* oxidoreductases. Ultimately, we hope that with these approaches, we will be able to identify new and vital targets in the fight against bacterial resistance.

FCT - Fundação para a Ciência e Tecnologia; MOSTMICRO ITQB

P50 | Mitotropic Radiocomplexes for Auger Therapy of Prostate Cancer

Santos, Joana (1); Braz, Maria Teresa (1); Silva, Francisco (1); Raposinho, Paula (1,2); Guerreiro, Joana (1); Cleeren, Frederik (3); Mendes, Filipa (1,2); Fernandes, Célia (1,2); Paulo, António (1,2)

(1) C2TN Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal;
(2) Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal;
(3) Laboratory for Radiopharmaceutical Research, Department of Pharmacy and Pharmacology, University of Leuven, Leuven, Belgium

Auger electron (AE) emitters hold great promise for targeted radionuclide therapy (TRT) of cancer, due to their high linear energy transfer over a nanometric range. When Auger emitters are internalized into highly radiosensitive organelles, such as the cell nucleus or the mitochondria, it is expected that the desired therapeutic effect is achieved with lower administered doses, thus minimizing side effects. Nuclear DNA has been considered the most relevant target of Auger electrons to have augmented radiotoxic effects and significant cell death. However, the mitochondria are recognized as one of the most important cellular targets to trigger apoptotic reactions and are also being studied as a subcellular target for therapeutic AE-emitting radionuclides[1,2].

In this context, we have designed dual-targeted ^{111}In-DOTA complexes carrying a Prostate Specific Membrane Antigen (PSMA) inhibitor ($PSMA_{617}$ derivative) and a triphenyl phosphonium (TPP) group to promote a selective uptake by prostate cancer cells and their accumulation in the mitochondria, respectively. Conjugates bearing a cathepsin B cleavable linker between the $PSMA_{617}$ moiety and the DOTA chelator were also synthesized, aiming at a further enhanced accumulation in the mitochondria upon enzymatic cleavage of the linker (Fig.1). In this way, we expected to obtain AE emitting radioconjugates suitable for a more selective TRT of metastatic castration-resistant prostate cancer.

In this communication, we will report on the synthesis and characterization of novel DOTA-based bifunctional chelators functionalized with $PSMA_{617}$ and TPP derivatives and on their respective indium complexes, obtained with ^{nat}In and ^{111}In. The preliminary biological evaluation of the radioactive ^{111}In-complexes was also performed to have a first insight on their potential usefulness for AE therapy of prostate cancer, and will be also presented. These biological studies included internalization and subcellular localization experiments in different cell lines (LNCaP, PC3-PIP and PC3-Flu), and the assessment of radiobiological effects based on the clonogenic survival assay.

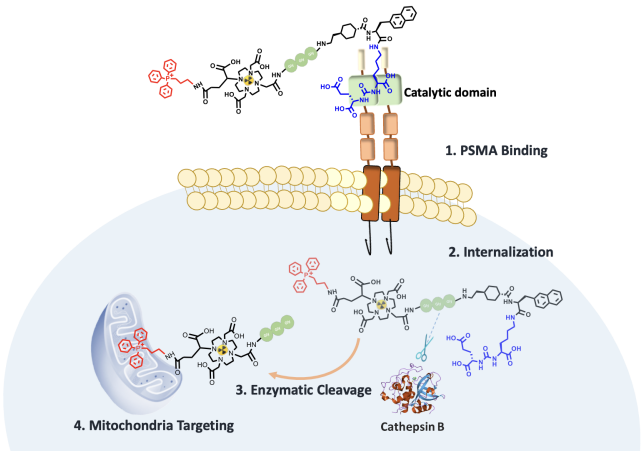

Figure 1: Schematic representation of the proposed strategy for the targeting of tumor mitochondria.

[1] Figueiredo, D et al. (2021) Molecules, 26(2), 441.
[2] Ku, A et al. (2019) EJNMMI Radiopharmacy and Chemistry, 4, 27.

This work was supported by Fundação para a Ciência e Tecnologia, Portugal (projects UID/Multi/04349/2019, PTDC /MED-QUI/1554/2020 and PTDC/BTM-TEC/29256/2017).

P51 | Evaluation of the correlation of oceanic water parameters considering the influence of sampling uncertainty

Borges, Carlos (1), Palma, Carla (2); Bettencourt da Silva, Ricardo (3)

(1) Chemistry and Biochemistry Department
(2) Instituto Hidrográfico
(3)Centro de Química Estrutural

Oceanic water masses present some features that distinguish them from transitional or freshwaters. These features are conservative oceanographic parameters like temperature and salinity. Previous studies suggest the existence of relationships/correlations between nutrients and some of these conservative parameters, e.g. [1]. However, the determination of this correlation is affected and can be masked by system heterogeneity and measurement (including sampling) uncertainty. This masking will be larger when large and heterogeneous systems are studied.

This work describes a tool to estimate the correlation between the values of a pair of parameters (applied to total oxidized nitrogen, NOx, and conductivity, C, in the present case) estimated from a large environmental area where the impact of system heterogeneity, sampling uncertainty and sample analysis uncertainty in the assessment is considered.

The uncertainty of "representative" sampling was estimated from the Monte Carlo simulation of georeferenced information affected by analytical uncertainty [2-3]. The quantification of the correlation between NOx and C was performed by Pearson's correlation coefficient, r. Each simulated pair of NOx and C values was obtained for the same GPS coordinates avoiding losing or reducing observed correlation from system heterogeneity. It was assumed that data correlation is meaningful if the calculated r is greater than the tabulated r value (rcrit) for a significance level, p, of 0.05 (i.e., for a 95% confidence level).

It was assessed the correlations between NOx, and, C, distinguishable regardless of system heterogeneity and analytical uncertainty.

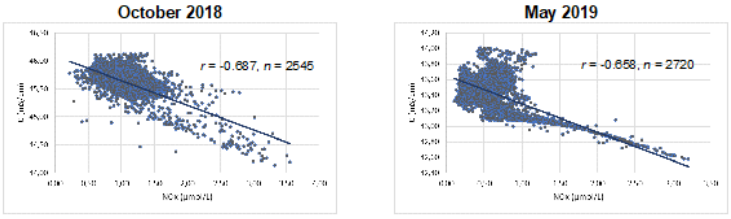

Figure 1: Correlations between NOx and C for the two sampling occasions (rcrit ≈ 0.040 for n = 2500).

[1] Iwata, T.; Shinomura, Y.; Natori, Y.; Igarashi, Y.; Sohrin, R.; Suzuki, Y., (2005). J. Oceanogr., 61, 721-732.
[2] Borges, C.; Palma, C.; Silva, R. B. (2019). Anal. Chem., 91, 5698–5705.
[3] Borges, C.; Palma, C.; Dadamos, T.; Bettencourt da Silva, R.J.N., (2021). Chemosphere, 263, 128036.

This work was financed by the Operational Program Mar2020 through project "AQUIMAR – Caracterização geral de áreas aquícolas para estabelecimento de culturas marinhas" and Fundacão para a Ciência e Tecnologia (FCT) through the multiannual financing programme 2020-2023 of Centro de Química Estrutural.

P52 | Extracellular regulation of tau aggregation and amyloid cross-interactions in Alzheimer's disease

de Freitas, Daniela P. (1), Moreira, Guilherme G. (1), Gomes, Cláudio M. (1)

(1) Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade Lisboa, 1749-016 Lisbon, Portugal. url: http://folding.fc.ul.pt

Despite being one of the main healthcare concerns of this century, an effective treatment for Alzheimer's disease (AD) has been elusive. Two main hallmarks characterize AD: a progressive neuroinflammation, and the appearance of proteinaceous aggregates, mostly composed by amyloid-β (Aβ) and tau. These proteins acquire aggregation-prone conformations that trigger their accumulation into oligomers and fibrils, finalizing in extracellular plaques, for Aβ, and intracellular neurofibrillary tangles, for tau. Even though these aggregates have a differential localization, tau exits the cell and spreads towards other brain regions in a pattern that correlates with the evolving brain damage during the disease. This highlights the extracellular space as an area where crucial events might be occurring, putting forward the possibility of an interaction between tau and Aβ which could be relevant for our understanding of AD development. Moreover, recent evidence points towards the earlier oligomers as being the main toxic species, emphasizing the need for studying the mechanistic process of amyloid formation and how it could be affected under different conditions. For this reason, we analysed the *in vitro* aggregation of tau, by using a new model known as the Tau AD core (TADC), which comprises the common ordered region of tau fibrils, which has been reportedly able to aggregate in the absence of cofactors1, increasing its similarity with *in vivo* settings. We have tested its effects on Aβ aggregation, noticing a potential inhibitory effect, while Aβ seems to exert an accelerating effect on TADC aggregation. Additionally, previous research in our laboratory has discovered that S100B, an abundant extracellular late-stage alarmin, has a chaperone-like activity, inhibiting Aβ and tau aggregation2. Indeed, when testing its effects on TADC, this inhibitory, concentration-dependent effect was evidenced. Further research is being developed to elucidate more of S100B's action on tau aggregation using a mixed model with Aβ.

[1] Camargo, D. R. et al. (2021). ACS Chem Neurosci. 12(23):4406-4415.
[2] Moreira, G. G. et al. (2021). Nat Commun. 12(1):6292.

P53 | Development of acid chars for microextraction of pharmaceutical compounds

Cerqueira, Jéssica (1); Neng, Nuno (1); Mestre, Ana.S (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

The present work aimed to develop and characterize acid chars as potential adsorptive phases for bar adsorptive microextraction technique (BAμE), followed by high performance liquid chromatography with diode array detection to monitor trace levels of nine pharmaceutical compounds and hormones in water matrices. Both polar and nonpolar compounds were selected as model compounds, such as, 17-α-ethinylestradiol, clofibric acid, mefenamic acid, carbamazepine, diclofenac, estrone, gemfibrozil, triclosan and sulfamethoxazole, to represent different therapeutic classes. The acid chars were prepared by sulfuric acid-mediated carbonization of sisal using two different concentrations, 9 M and 13.5 M. To study the potential of acid chars as BAμE phase, the effect of different organic solvents to clean the acid chars and the influence of the matrix pH were performed.

For benchmarking the two lab-made acid chars were tested along with a commercial powdered activated carbon. The carbon materials (acid chars and commercial activated carbon) were characterized by nitrogen adsorption isotherms and by the pH at the point of zero charge. The results obtained showed that, despite having incipient porosity, the acid chars managed to obtain a response for all analytes under study and in some cases, the recoveries efficiencies overcame those attained with the commercial activated carbon used as control. Therefore, this work showed that acid chars have potential as adsorbents for samples enrichment.

P54 | Tissue tropism and parasite virulence in recently isolated Trypanosoma brucei strains

Valido Narciso, Marta (1); Trindade, Sandra (1); Silva Pereira, Sara (1); M. Figueiredo, Luísa (1)

(1) Instituto de Medicina Molecular - João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Trypanosoma brucei is a unicellular and extracellular parasite responsible for African Trypanosomiasis in humans and cattle. In experimental infections, researchers typically use the EATRO1125 strain (clone AnTat 1.1E 90:13), a laboratory-adapted pleomorphic strain, in which parasites can differentiate from the replicative "slender" to the cell-cycle arrested "stumpy" form. In the mammalian host, besides circulating in the blood, trypanosomes invade tissues. Recently, two new strains of *T. brucei brucei*, MAK65 and MAK98, were isolated from Ugandan cattle. [1] In this study, we asked how similar was the behavior of these recently isolated T. brucei strains relative to the EATRO1125 strain *in vitro* and *in vivo* . In *in vitro* cultures, the doubling time of MAK65 was approximately two-times lower than MAK98 (6h vs 12,5hr, respectively), which agrees with recent literature. [1] Consistently, survival experiments in C57BL/6J or BALB/cByJ mice showed that MAK65 is slightly more virulent than MAK98. Independently of the host, both natural strains led to hyper parasitaemia in the blood resulting in mice death within the first two weeks of infection, whereas EATRO1125 led to chronic disease (mice die after day 30).

To understand the reason why parasitemia levels are so high, we will extract RNA from MAK65/98 parasites on day 5/6 post-infection in C57BL/6J mice to assess whether these naturally isolated strains can differentiate to stumpy forms by performing qPCR of stumpy-marker genes. Tissue tropism was assessed by qPCR in major organs, and we observed that in previously identified major extravascular reservoirs, the pancreas [2] and gonadal adipose tissue [3], the MAK98 strain behaves similarly to EATRO1125. Our study highlights the importance of using multiple strains when analyzing infection phenotypes as pathology can be extremely dependent of parasite strain.

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P55 | A computational method to estimate pH effects in antitumor drug resistance

Suzano, Pedro (1) Silva, Tomás (1) Machuqueiro, Miguel (1)

(1) BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Many antitumor drugs cross the lipid membrane by passive diffusion to enter tumor cells. In the case of weak (Lewis) base drugs, which are charged in water (pKa values are generally between 8 and 9), a transient deprotonation is required to cross the lipid bilayer [1]. Since the tumor microenvironment (TME) is slightly more acidic than normal cells, it has been proposed that this increased acidity can decrease the antitumor efficiency of these Lewis bases by impairing the transient deprotonation process [1].

To quantify the impact of the TME in the membrane permeability of some chemotherapeutics, we propose a new protocol based on Constant-pH Molecular Dynamics [1] coupled with an Umbrella Sampling scheme (US-CpHMD) [2] and applied it to two tyrosine kinase inhibitor drugs, sunitinib and nintedanib, interacting with a POPC lipid bilayer. The membrane permeability coefficients were calculated using the inhomogeneous-solubility diffusion model (ISDM) [3]. The calculations were performed at different pH values, namely 7.5 to mimic a healthy cell, 6.0 to model the TME acidity, and 4.5 to capture the strong acidity of the lysosomes lumen. The latter can provide some insights on the lysosomal sequestration phenomenon, which has been proposed as a drug resistance mechanism [1]. We have calculated the impact of acidity in the bioavailability of both sunitinib and nintedanib, which will help us design new compounds that should be able to circumvent these limitations.

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P56 | Monitoring the optical properties and swelling of low temperature synthesized PEDOT:PSS films during redox conversion

Santos, Daniel R. (1); Zeferino, Jorge (1); Viana, Ana S. (1); Wijayantha, Upul (2); Lobato, Killian (3); Correia, Jorge P. (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;
(2) Centre for Renewable Energy Systems, Cranfield University, Cranfield, Bedford MK43 0AL, UK;
(3) Instituto Dom Luiz, FCiências, ULisboa, Campo Grande, 1749-016 Lisboa, Portugal

Lithium-ion batteries are, nowadays, the energy storage system with the most widespread use but, at this moment, there are insufficient lithium resources, consequently causing an unbalanced demand for this metal [1]. In terms of availability of raw material resources, sodium-ion batteries appear as a promising alternative. This change implies the design of new cathode materials capable of accommodating a larger ion [2]. The cathodic structure must be chemically stable and extremely light [3] to balance the mass increase of the storage unit, imparted by the lithium replacement by sodium. An electronically conducting polymer with pseudocationic doping character may fulfil these requirements. In this work poly(3,4-ethylenedioxythiophene) doped with poly(styrene-4-sulfonate) (PEDOT:PSS) films were galvanostatically synthesized at 0^{o}C for different time intervals (10 s - 50 s) on platinum electrodes from aqueous solutions containing the monomer and the polyanion.

A proper and uncommon strategy was applied to assess the polymer optical properties and thickness variation as a function of the potential applied, during redox conversion. Therefore, the redox behaviour of films synthesized with different growth charges in aqueous medium, was monitored by cyclic voltammetry at room temperature in $NaClO_4$ containing acetonitrile solution, with simultaneous recording of ellipsometric parameters. The plotting of psi vs. delta of the different films at selected potential values, allowed to simulate the films growth effect on the dielectric constants at different degrees of doping, as a function of their thickness. The optical constants obtained allow accessing the polymer properties imparted by the low temperature synthesis, as well as the swelling/shrinking behaviour related to the pseudocationic character of the doping process. Further support of the adequacy of the model used to compute the relevant ellipsometric information was provided by in-situ UV-Vis reflectance spectrophotometry experiments.

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P57 | Natural Deep Eutectic Systems a new delivery system for ocular drugs

Sarmento, Célia (1); Paiva, Alexandre (1); Duarte, Ana Rita C. (1); Jesus, Ana Rita (1)

(1) Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, Portugal

Worldwide, more than 2 billion people suffer from vision impairments or blindness due to ocular diseases. Cataracts are the most prevalent, followed by glaucoma, diabetic retinopathy and age-related macular degeneration (ARMD).The major causes of these ocular diseases are the natural aging process and the photooxidation caused by daily light exposure.

The most common therapeutic strategies include drops, ointments, injections and surgery. But drops are the most practical and patient-compliant way to treat ocular diseases. However, they pose many challenges regarding their formulation, since many drugs are insoluble and unstable presenting low bioavailability.[1]

Recently, natural deep eutectic systems (NADES) have emerged as alternative systems for many applications. NADES are described as a mixture of two or more components, which at a particular composition present a high melting point depression, compared to its individual constituents, becoming liquid at room temperature. NADES are composed by natural metabolites such as sugars, amino acids, and polyols, hence biocompatible; NADES are biodegradable, task-specific, and cost-effective since no waste is produced nor requires purification steps.[2] NADES are known to easily dissolve poorly water-soluble drugs and to stabilize biomolecules.[3] Moreover, NADES present high viscosity that in this application is actually a key feature because most ocular formulations contain polymers, called viscosity enhancers, to increase the retention time of the drug in the eye surface. Therefore, the use of NADES as ocular drug delivery systems would allow to significantly decrease the use of those polymers. In this work we took the advantage of nature to develop greener and more sustainable ocular delivery systems, based on NADES. Rheological studies on NADES show that they can be used as ocular drug delivery systems.

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P58 | Cannabis Consumption in the University Population in Portugal: Determination of Biomarkers in Hair and Oral Fluid Samples

Antunes, Mónica (1); Fonseca, Suzana (2); Simões, Susana (2); Franco, João (2); Gallardo, Eugenia (1); Barroso, Mário (2)

(1) Centro de Investigação em Ciências da Saúde, Laboratório de Fármaco-Toxicologia, UBIMedical, Universidade da Beira Interior, Covilhã, Portugal
(2) Serviço de Química e Toxicologia Forenses, Instituto Nacional de Medicina Legal e Ciências Forenses, I.P., Delegação Sul, Lisbon, Portugal

Over 96 million European adults are estimated to have consumed illicit drugs at some point in their lives. Cannabis is still the most consumed drug, being estimated that 27.4 % of European young adults have used cannabis in 2018 [1], while in Portugal a percentage of 11 % is referred for 2016. Also, 15 % of students aged 15 to 16 have used this drug at least once. This Portuguese data was obtained using surveys [2], but this type of study has several disadvantages, such as under or overestimation of consumption rates, not necessarily on purpose, which may lead to biased conclusions. Consequently, it is desirable that these studies are accompanied by drug monitoring in biological samples to circumvent the associated drawbacks.

Thus, this study aims at evaluating cannabis consumption amongst Portuguese university students by hair and oral fluid testing. These specimens' collection procedures are non-invasive, and they provide information concerning mid to long-term and recent exposure to drugs (from hair and oral fluid analysis, respectively). The analytical methods are being developed and validated according to the guiding principles of the Scientific Working Group for Forensic Toxicology (SWGTOX) and the Society of Hair Testing (SoHT) for representative cannabis drugs (THC, THC-OH and THC-COOH) and two components of medicinal cannabis (CBN and CBD), to differentiate between consumption modes. The developed methods will be applied to samples collected from students attending Portuguese universities, and the results will be compared to data obtained from self-completion questionnaires, providing more accurate estimations of consumption rates in the studied population. The information gathered in this project will be a good starting point for a comparison with the reported values in official statistical bulletins. Furthermore, the obtained results will serve as a basis to identify risky groups and possible targets for preventive measures and public policies to manage drug abuse.

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P59 | Theranostic potential of $^{64}CuCl_2$ in three-dimensional cell culture models of prostate cancer

Pinto, Catarina I.G. (1) Bucar, Sara (2) Fonseca, Alexandra (3) Alves, Vitor (3) Abrunhosa, Antero J. (3) da Silva, Cláudia L. (2) Guerreiro, Joana F. (1) Mendes, Filipa (1,4)

(1) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal;
(2) Departamento de Bioengenharia, iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal;
(3) CIBIT/ICNAS Instituto de Ciências Nucleares Aplicadas à Saúde, Universidade de Coimbra, Coimbra, Portugal;
(4) Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.

Prostate cancer (PCa) remains the second most common cancer type in men, and it is still considered incurable in advance stages. This justifies the need to ensure an early diagnosis and develop new approaches that can surpass this cancer's chemoresistance. Radiopharmaceuticals are excellent tools for this challenge as they have the potential to be used in diagnosis, therapy or theranostics. In particular for PCa, we have previously demonstrated, using monolayer-cultured cells, that copper-64 chloride ($^{64}CuCl_2$) has the potential to induce damage in PCa cells, having minimal side effects in non-tumoral cells, being a promising theranostic agent for PCa. [1] However, considering the limited predictive value of the monolayer-cultured cells model, the results obtained may not always be translatable *in vivo* .

Here, we further assessed the theranostic potential of $^{64}CuCl_2$ using more advanced PCa cellular models, namely multicellular spheroids. [2] Spheroids are 3D culture models that can better replicate the metabolic and proliferative gradients of *in vivo* tumors. Unlike monolayer cultures, spheroids have an increased population of cancer stem cells (CSCs), important contributors to increased tumor resistance and recurrence. After the initial establishment and characterization of the growth behavior and CSCs population of three PCa spheroids, derived from 22RV1, DU145 and LNCaP PCa cell lines, we assessed the cellular uptake of $^{64}CuCl_2$, and evaluated changes in the growth profile and viability of the spheroids exposed to $^{64}CuCl_2$, as well as the clonogenic capacity of spheroid-derived cells. The results obtained showed that $^{64}CuCl_2$ is able to significantly reduce the spheroids' growth and viability, as well as their reproductive ability. Of notice, spheroids with the highest initial percentage of stem-like cells were found to be the most resistant to $^{64}CuCl_2$ treatment. Altogether, the results obtained confirmed the high potential of $^{64}CuCl_2$ as a theranostic agent for PCa.

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P60 | Characterization of noncovalent interactions in biomolecular simulations for drug discovery improvement: using a robust and unsupervised energy-based criterion

Dias, Tiago (1) Victor, Bruno (1) Costa, Paulo (1)

(1) Chemistry and Biochemistry Department

Noncovalent interactions, such as hydrophobic interactions, hydrogen and halogen bonds, salt bridges, and aromatic stacking, are essential in structural biochemistry, drug discovery, and biology [1]. Automatic tools for assigning the presence of such interactions in 3D structures (e.g. PDB, molecular dynamics trajectories) are extremely useful in drug design since, in the early stages of this process, a comparative analysis of binding patterns for a given target is frequently performed [1]. These tools commonly use geometric criteria to assign interactions such as hydrogen or halogen bonds, often using angle and distance thresholds. This method, however, is empirical and arbitrary and might erroneously include or exclude interactions.

Therefore, in this work, we aim to develop a robust and unsupervised energy-based criterion, inspired by the popular method Define Secondary Structure of Proteins (DSSP) algorithm, to assign noncovalent interactions in biomolecular systems. Based on previous work done by the group [2,3] in this communication we present the development of a fast computational workflow implementing an energy-based criterion for the identification of non-covalent interactions in protein-ligand or membrane-ligand MD simulations. This method relies on the estimation of the probability density function which can be applied to a 2D descriptor system (distance, angle) or to a 1D descriptor (potential energy). The latter is unprecedented and is a fast and accurate method for bond assignment. With this work, we hope to improve drug discovery tools.

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P61 | Impact of the halogen radii in the determination of binding free energies using MM-PBSA calculations

Fortuna, Andreia (1,2); Costa, Paulo J. (1)

(1) BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa and Chemistry and Biochemistry Department
(2) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon

Although several drug and drug-like molecules are halogenated, empirical force fields typically consider halogen atoms to carry a negative charge leading to unfavorable interactions with Lewis bases, thus omitting the ability to establish halogen bonds. These arise from a positive region on the electrostatic potential of these elements (σ-hole) and an efficient way to overcome this problem is to introduce an off-center point charge(EP) in those force fields. MM-PBSA calculations rely on molecular dynamics trajectories, which could be generated using an EP, and allow the determination of binding free energies (ΔGbind) with a low computational cost, which is important for large libraries of compounds. The solvation free energy is estimated using Poisson–Boltzmann Surface Area (PBSA) calculations which depend , among other parameters, on the empirical assignment of atomic radii (PB radii). Recently, the optimization of halogen atomic radii for PBSA calculations, using off-center point charges, resulted in a significant decrease in the mean absolute errors (against experimental values) of calculated solvation free energies of halogenated molecules [1]. However, the impact of these radii on the determination of ΔGbind remains unknown. Protein kinase CK2 complexes are suitable for the study of halogen bonds since tetrabrominated benzimidazole derivatives are a well-known class of CK2 inhibitors which can establish at least two halogen bonds between the bromine atoms and the oxygen atoms of Glu114 and Val116. Moreover, the calculation of ΔGbind of these complexes has been assessed previously with PM6-DH2X [2] and MM-GBSA [3] calculations, the latter using the AMBER force field with the EP methodology but with non-optimized radii. In this study, the impact of the optimized halogen radii is explored by performing MM-PBSA calculations using various EP implementations and three different approaches - using the X-ray structure, an FF-minimized structure, or a set of conformations sampled from MD simulations.

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P62 | Impact of sphingolipid profile on yeast gel domains and membrane compartments

Bento-Oliveira, Andreia(1); C. Santos, Filipa(1); T. Marquês, Joaquim(1); M.R. Paulo, Pedro (2); Korte, Thomas(3); Herrmann, Andreas(3); Susana Marinho, H.(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
(2) Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal
(3) Institut für Chemie und Biochemie, Freie Universität Berlin, Germany

There are two major compartments in the plasma membrane of yeast *Saccharomyces cerevisiae* - membrane compartment containing the arginine permease Can1p (MCC), and membrane compartment containing the H^+-ATPase Pma1p (MCP) [1]. In yeast plasma membrane, sterol-rich domains (SRDs) are distinct from sphingolipid-enriched domains (SLEDs), which are ergosterol-depleted, and biophysically unique, as they are highly rigid gel domains [2]. The interplay between these domains and yeast membrane compartments is still unclear.

To tackle this question, we evaluated how the hydroxylation pattern or polar headgroup of complex sphingolipids impacts membrane biophysical properties, particularly SLEDs and SRDs, and Pma1p and Can1p distribution and microenvironment. To achieve this, *S. cerevisiae* cells, wild-type and two mutants, one lacking the main yeast complex sphingolipid M(IP)2C (ipt1delta) and the other with sphingolipids lacking acyl chain 2-hydroxylation (scs7delta) were compared. Tighter packing of SLEDs, with a tendency for lower abundance, was found in both mutants when compared to the wt. However, no significant alterations could be perceived in ergosterol-enriched domains. Concomitantly, the distribution of Pma1p along the plasma membrane was considerably more heterogeneous in the mutant cells, while Can1p did not exhibit noticeable alterations. Moreover, the microenvironment surrounding Can1p was not affected by changing the sphingolipid profile, while the opposite seems to occur for Pma1p [3]. Since MCP and MCC have been described as spatially separated, the results obtained in this work support an independent compartmentalization of sphingolipid-enriched and sterol-enriched domains, and that these regions could be lipid domain counterparts of the MCP and the MCC, respectively.

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List of Participants

Adhan Pilon - adpilon@fc.ul.pt
Alexandre Filipe dos Reis Coelho - afdcoelho@fc.ul.pt
Ana Catarina Carvalho da Rocha - fc55592@alunos.fc.ul.pt
Ana Clara Brito Ferreira - acb.ferreira@campus.fct.unl.pt
Ana Paula Pereira Paiva - appaiva@ciencias.ulisboa.pt
Ana Rita da Silva Santos - ana.rita.santos@tecnico.ulisboa.pt
Ana Rita Jesus - ar.gameiro@fct.unl.pt
Ana Rita Pinheiro - anar.pinheiro@gmail.com
Ana Rita Rodrigues Reis - ar.reis@campus.fct.unl.pt
Ana Roda - a.roda@fct.unl.pt
Ana Silveira Viana - anaviana@fc.ul.pt
Ana Sofia Lucas Silva Reis Augusto - sofiaagusto@gmail.com
Ana Sofia Martins Rio - rio.sofia29@gmail.com
André Filipe Batista Mendes - andre.fb.mendes99@gmail.com
Andreia Bento de Oliveira - abdoliveira@fc.ul.pt
Andreia Cristina Janeiro - fc54673@alunos.fc.ul.pt
Andreia Fortuna - ajfortuna@fc.ul.pt
André Miguel Vaz Pinto Santos - andre.m.santos@medicina.ulisboa.pt
António José de Jesus Matos Figueira - ajfigueira@fc.ul.pt
Bárbara Bruni - bah.bruni@gmail.com
Bárbara Joana de Almeida Henriques - bjhenriques@fc.ul.pt
Beatriz Lopes Columbano Almeida - beatrizcolumbanoalmeida@gmail.com
Beatriz Lopes - fc57168@alunos.fc.ul.pt
Bruna Filipa Santos - santosbrunaf@hotmail.com
Bruno Emanuel de Sá Calçada - fc34515@alunos.fc.ul.pt
Bruno Lourenço da Silva Victor - blvictor@fc.ul.pt
Carlos Manuel Barata da Fonseca Borges - carlos.borges@hidrografico.pt
Carolina Ruivinho - csruivinho@fc.ul.pt
Carolina Sequeira - carolina.margarido@hotmail.com
Catarina Barradas Antunes Maria - c.maria@campus.fct.unl.pt
Catarina Isabel Guilherme Pinto - catarinaigpinto@gmail.com
Catarina Pereira do Nascimento - catarina_nascimento98@hotmail.com
Catarina Seixas Caldeira - catarina.caldeira@sapo.pt
Cátia Sofia Duarte Lopes - csolopes@fc.ul.pt
Célia Cristina Oliveira Sarmento - cc.sarmento@campus.fct.unl.pt
Cláudia Alexandra da Silva Rodrigues - cadrodrigues@fc.ul.pt
Cláudio Correia Fernandes - cco.fernandes@campus.fct.unl.pt
Cláudio M. Gomes - cmgomes@fc.ul.pt
Cristiana Andreia Sousa Vilela - cristianasvilela@gmail.com
Cristiana Isabel Martins Ferreira - cristiana.ferreira@tecnico.ulisboa.pt
Daniela de Freitas - daniela.plasencia.df@gmail.com
Daniela Filipa Ribeiro Ferreira - ferreira-daniela@edu.ulisboa.pt
Daniel Matias - dfmatias@fc.ul.pt
Daniel Rúben Costa Reis Santos - drcsantos@fc.ul.pt
David Fernandes das Neves Pereirinha Ramalho - fc53713@alunos.fc.ul.pt
David Miranda - fc53075@alunos.fc.ul.pt
Diana Isabel Marques Vitorino - dianavitorino17@gmail.com
Diogo dos Santos Baptista - diogosb1108@gmail.com
Duarte Antunes - duartemantunes@gmail.com
Duarte Breia Clemente - duarteclemente@alunos.fc.ul.pt
Edite Ferreira Pinto - editefpinto@gmail.com
Filipa Sofia Libório Carvalho - fslcarvalho@fc.ul.pt
Filipe Eduardo Pequito Rodrigues - ferodrigues@fc.ul.pt
Filipe Silva Nunes de Oliveira - fsn.oliveira@campus.fct.unl.pt
Filomena Martins - filomena.martins@fc.ul.pt
Flávia Fátima Cunha Rodrigues - flavia.f.cunha@hotmail.com
Francisco Maria Reis Ventura Rosado Traquete - fmtraquete@fc.ul.pt
Francisco Pinto - frpinto@fc.ul.pt
Gabriel Frederico Ferreira Martins - gfmartins@fc.ul.pt
Gonçalo Branco Guimarães Maia - gossassini@gmail.com
Gonçalo Mendes Gilberto - goncalo.gilberto@gmail.com
Gonçalo Miguel Pinto Batista e Sá Vieira - gm.vieira@campus.fct.unl.pt
Gonçalo Santos Moura Trindade - goncalo.trindade@ibet.pt
Guilherme Gil da Silva Veríssimo Moreira - ggmoreira@fc.ul.pt
Hugo Filipe Nunes Monteiro - h.monteiro@campus.fct.unl.pt
Inês Alexandra de Sá Martins - samartins.ines@gmail.com

Inês Domingos Silva Pires - idpires@fc.ul.pt
Inês Margarida Martins Alves - inesalves03@gmail.com
Ines Pankonien - ipankonien@fc.ul.pt
Inês Vieira Peres Ventura - inesventura93@gmail.com
Israa Alakhras Aljnadi - israa.aljnadi@campus.ul.pt
Jéssica dos Santos Ribeiro de Freixo Cerqueira - fc53052@alunos.fc.ul.pt
Jéssica Irina Pisco Cabrita - jessica.irina@gmail.com
Joana Filipa da Silva Santos - joana.f.santos@tecnico.ulisboa.pt
Joana Filipa Portugal da Rocha Terreiro - jterreiro1@gmail.com
Joana Valente Ribeiro - mjvribeiro@fc.ul.pt
João André Isidoro Miranda - jamiranda@fc.ul.pt
João Gonçalo Nunes Sequeira - jgsequeira@ciencias.ulisboa.pt
João Luz - joaomluz97@gmail.com
João Miguel Franco Machado - jmfmachado@fc.ul.pt
João Nuno Marques Vitorino - jnvitorino@fc.ul.pt
Jorge Correia - jorge.correia@fc.ul.pt
Jorge Fernandes da Silva João - jorgejoao@tecnico.ulisboa.pt
Jorge Filipe Araújo de Antunes Zeferino - jorgefzeferino@alunos.fc.ul.pt
José Júlio Pereira Gantes da Costa - jliuze@gmail.com
Luís C. Almeida - lmalmeida@fc.ul.pt
Marco Alexandre Pina de Sá - fc53051@alunos.fc.ul.pt
Margarida Soeiro Martins - margaridamartins.9920@gmail.com
Margarida Veloso - mveloso@medicina.ulisboa.pt
Maria de Sousa Gama - mariadesousagama00@gmail.com
Mariana Dias Machado - fc55810@alunos.fc.ul.pt
Mariana Mendes - marianamendes07@live.com.pt
Mariana Vieira Pereira - fc49731@alunos.fc.ul.pt
Marta Sofia Pedro Batista - martaspbat@gmail.com
Marta Sofia Silva da Cruz Botelho - fc53673@alunos.fc.ul.pt
Marta Valido Narciso - martavalidonarciso@gmail.com
Miguel Barbosa Louro dos Santos - mbl.santos@campus.fct.unl.pt
Miguel Machuqueiro - machuque@ciencias.ulisboa.pt
Miriam Raposo Colaço - mr.colaco@campus.fct.unl.pt
Mónica Sofia Soares Antunes - antunes.ss.monica@hotmail.com
Neuza Inês Guerreiro Salgado - neuzaines@hotmail.com
Nishta Ramkhelawon - nishtasramkhelawon@gmail.com
Nuno Filipe Baltazar Maia Costa de Oliveira - nfoliveira@ciencias.ulisboa.pt
Nuno Galamba - njgalamba@fc.ul.pt
Oriana Carolina Gonçalves Pestana - ocgoncalvesp@alunos.fc.ul.pt
Paulo N. Martinho - pnmartinho@fc.ul.pt
Paulo Rodrigo Coelho Lopes - fc54348@alunos.fc.ul.pt
Pedro Miguel Sousa Suzano - pmsuzano@fc.ul.pt
Pedro Rafael Magalhães - prmagalhaes@fc.ul.pt
Rafaela Tenera Marques - rfmarques@fc.ul.pt
Rafael Santana Nunes - rsnunes@fc.ul.pt
Ricardo Jorge Gonçalves Teixeira - rjteixeira@fc.ul.pt
Ricardo Jorge Marques de Lemos - ricardo_de_lemos@outlook.pt
Ricardo Lima Navarro Silva de Oliveira - rl.oliveira@campus.fct.unl.pt
Ricardo Silva - rsricardo.1009@gmail.com
Rita Isabel Carvalho Teixeira - ri.teixeira@itqb.unl.pt
Rodrigo Miguel de Sousa Barriga Alves - rodrigoalves.esss@gmail.com
Sara Alexandra Barros da Gama - sgama@medicina.ulisboa.pt
Sara Alexandra Peça de Sousa Rosa - sarasousarosa@tecnico.ulisboa.pt
Sara Angélica Alves Oliveira Badaró - sara.badaro@edu.ulisboa.pt
Sara Gabriela Ferraz Ferreira - sferreira@fc.ul.pt
Sofia Alexandra de Albuquerque Martins - sada.martins@campus.fct.unl.pt
Sofia Maria Sousa Daniel - fc54698@alunos.fc.ul.pt
Sónia Filipa Gomes dos Santos - sonia.santos@northumbria.ac.uk
Susana Catapirra Magessi Parreiras - s.parreiras@campus.fct.unl.pt
Tânia Melizia Pereira Moreira - taniamelizia1998@gmail.com
Tânia Morais - tsmorais@fc.ul.pt
Tiago Alexandre Duarte Delgado - tiago.duarte.delgado@gmail.com
Tiago Gonçalves Dias - tiagogdias98@gmail.com
Tiago Pereira Gomes - tpereiragomes@hotmail.com
Tomás Silva - tfsilva@fc.ul.pt
Vanessa Morgado - vmmorgado@fc.ul.pt
Vanessa Sofia da Silva Esteves - fc56076@alunos.fc.ul.pt
Vasco Miguel Candeias Cachatra - vmcachatra@fc.ul.pt
Vladimir Ghilas - ghilasvladimir@gmail.com

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