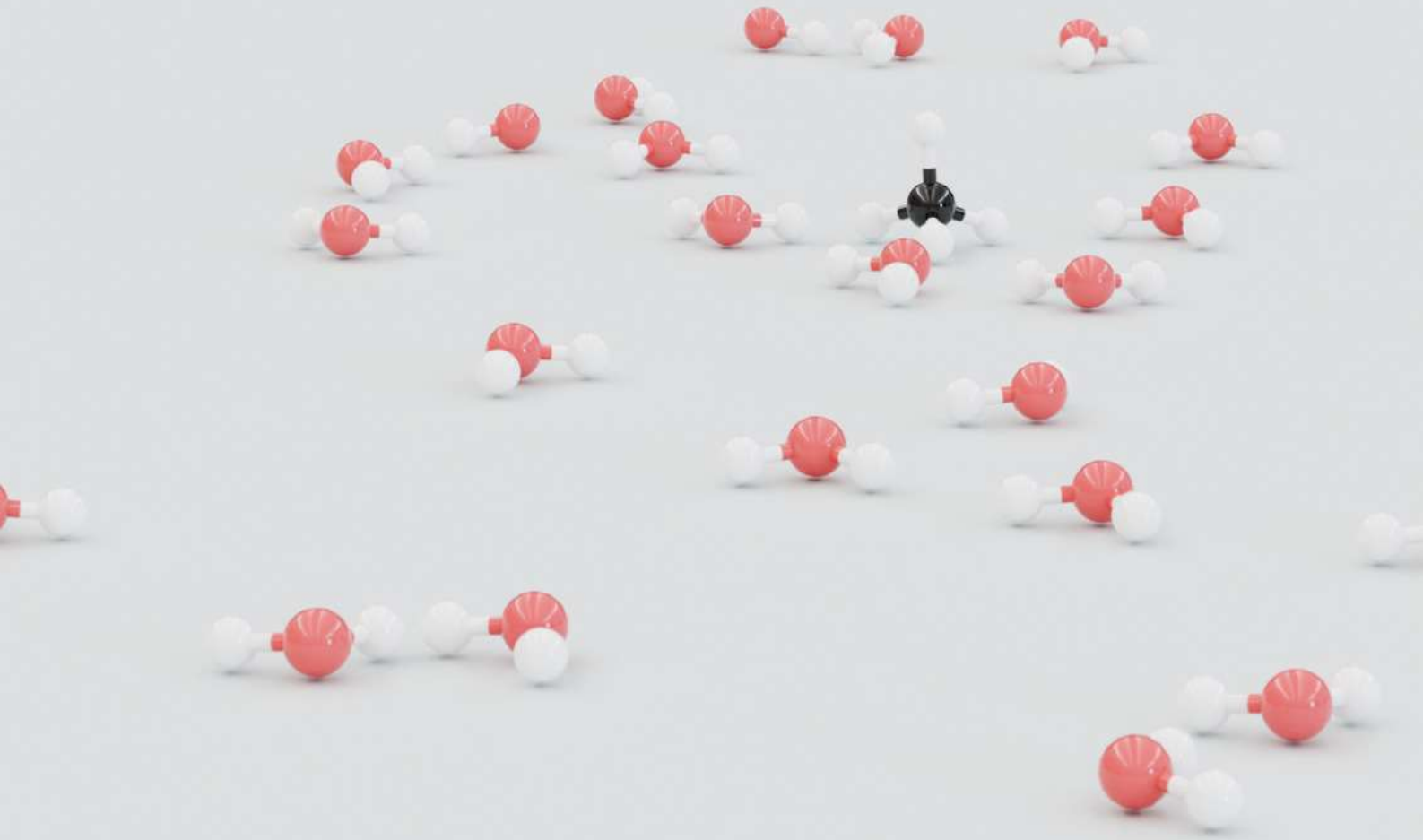
BOOK OF ABSTRACTS

4th Chem & Biochem Students Meeting



June 27th, 2024

4th Chem & Biochem Students Meeting

Book of Abstracts

Preliminary

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

General index

Plenaries

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

Unlocking The Seaweed Potential for different Biotechnological Applications

Pinteus, Susete (1,2)

(1) MARE; (2) IPLeiria

Seaweeds are a treasured natural resource that continues to be scarcely explored. Thanks to their ability to produce bioactive compounds, with unique chemical structures, seaweed can have a wide range of biotechnological applications, from therapeutics to cosmetics, food, and maritime industry, among others. MARE-IPLeiria has a strong research group working in blue biotechnology which is focused on the extraction, screening and isolation of marine bioactive compounds, having successfully identified numerous chemical structures with significant potential across various industries, addressing the growing societal demand for natural, sustainable, and more effective products. This communication will disclosure the biotechnological potential of seaweed to deliver bioactive compounds for i) skincare (anti-ageing, photoprotection, anti-acne); ii) therapeutics (neuroprotective and antitumoral); iii) food (antimicrobial, antioxidant); and iv) maritime industry (antifouling capacity). As scientific exploration continues, seaweed is poised to reveal even more groundbreaking applications, cementing its role as a key resource in future biotechnological advancements.

A novel link between Type-2 Diabetes mellitus and Parkinson's disease: The role of glycation and insulin-degrading enzyme dysfunction

Chegão A. (1), Sousa L. (1), Vicente Miranda H. (1)

(1) NOVA Medical School, Universidade NOVA de Lisboa

Parkinson's disease (PD), the second most prevalent neurodegenerative disease, remains incurable and debilitating. Most cases are sporadic, complicating etiological identification. Type-2 Diabetes mellitus (T2DM) is a significant risk factor, with T2DM patients having a 40% higher risk of PD, rising to 280% in those aged 25-45 years. Our research focuses on how T2DM contributes to PD pathogenesis, particularly through hyperglycemia and increased protein glycation.

Using animal models, we found that brain glycation worsens or initiates motor, cognitive, olfactory, and colonic impairments in PD mice, providing the first evidence that increased brain glycation can cause PD. Proteomics revealed that mitochondrial function and protein quality control are compromised in glycation conditions. Additionally, glutamatergic signaling proteins are increased in regions with dopaminergic neurons, suggesting glycation-induced glutamatergic excitotoxicity as an early PD mechanism.

We also observed that diabetes leads to a loss of Insulin-Degrading Enzyme (IDE) in the brain. We are exploring the impact of IDE failure in both IDE knockout (KO) and PD mice under standard or hypercaloric diets, which induce prediabetes. Preliminary findings suggest that IDE KO induces impaired motor performance, and that diabetes exacerbates PD-like phenotypes.

These findings highlight new therapeutic targets for PD, suggesting that preventing brain glycation and addressing IDE dysfunction could prevent PD development in T2DM patients and slow PD progression in PD patients.

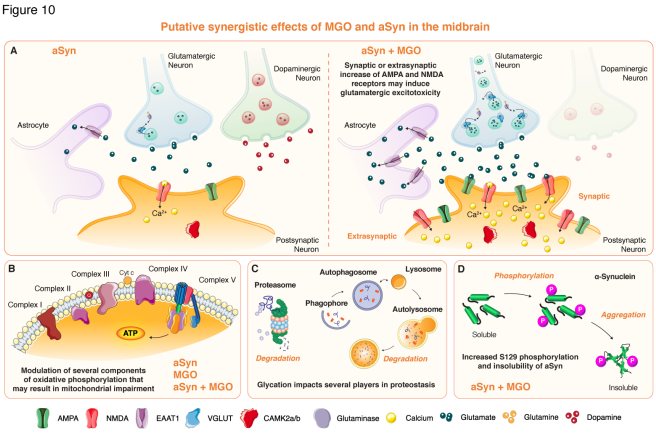


Figure 1: Putative synergistic effects of MGO and aSyn in the midbrain.

References

[1] Chegão A, Vicente Miranda H. Behav Brain Res. 2023 Mar 28; 442:114309 [2] Chegão A, ..., Vicente Miranda H. npj Parkinson's disease, 2022 Apr 25;8(1):51. [3] Sousa L, ..., Vicente Miranda H. J Pathology, 2021 Dec;255(4):346-361.

Acknowledgements

Michael J. Fox Foundation for Parkinson's research Fundação para a Ciência e Tecnologia Sociedade Portuguesa de Diabetologia

Oral Communications

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

OC1 - Structural biology and biophysical analysis of the EARS2 protein and disease variants: unveiling their role in Leukoencephalopathy

Dias, A. A. (1,2); Ribeiro, J. V. (1,2); Gomes, C. M. (1,2); Henriques, B. J. (1,2)

(1) - BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal. (2) - Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Aminoacyl-tRNA synthetases (aaRS) proteins are responsible for adding an amino acid to the corresponding tRNA and, consequently, they are indispensable for the translation process in the cell. Interestingly, mutations in genes encoding for mitochondrial aaRS (mt-aaRS) have been associated to particular mitochondrial disorders (MD), and an increasing number of protein variants are being identified, including in Portugal. Hence, it is essential to clarify the molecular mechanisms behind these MDs and, in particular, to provide information on mt-aaRS structure, conformation, and function to decipher the impact of the identified mutations. We aim to contribute to the field by studying the mitochondrial glutamyl-tRNA synthetase (EARS2), employing biochemical and biophysical methods to make for the first time, to our knowledge, a structural characterization of the human EARS2 wild-type and three disease variants associated with leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL). We have heterologously expressed and purified the EARS2 wild-type in E. coli, achieving a purity yield higher than 90%. Analysis of secondary and tertiary structure revealed that the purified protein presents a folded conformation, and an apparent melting temperature of 45℃. Regarding disease variants, we established protocols using co-expression with molecular chaperones to increase the bacterial expression yield. We are also optimizing the purification of the EARS2-p.E96K variant. We believe that implementing the purification protocol for this mt-aaRS will open new avenues for characterizing variants and, in the future, aid in designing disease therapies.

References

Acknowledgements

Fundação para a Ciência e Tecnologia (FCT/MCTES, Portugal) for grants: Scientific Employment Stimulus-Individual Call 2021.02218.CEECIND (DOI: 10.54499/2021.02218.CEECIND/CP1650/CT0008) (to B.J.H), SFRH/BD/147202/2019 (DOI: 10.54499/SFRH/BD/147202/2019) (to J.V.R.), PTDC/BIA-BQM/29963/2017 (DOI: 10.54499/PTDC/BIA-BQM/29963/2017) (to B.J.H), centre grants UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/MULTI/04046/2020 (DOI: 10.54499/UIDP/04046/2020) (to BioISI) and an initiation to investigation fellowship (BII) under the BioISI Junior Programs (A.A.D.). This research is partially funded by the European Union (TWIN2PIPSA, GA 101079147).

OC2 - Carbon dioxide conversion with Metal-Organic Frameworks

Ferreira, M. (1); Realista, S. (1); Martinho, P. (1)

(1) - Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, 1749-016 Lisboa, Portugal.

Over the last few decades, the greenhouse effect, which is crucial to our survival, has become a risk. The increase of the concentration of greenhouse gases (e.g. CO_2, CH_4, etc) in the atmosphere is leading to a rise in the Earth's average temperature, impacting various ecosystems. Carbon dioxide is one of the gases that most contributes to this effect. Consequently, many catalytic methods for converting CO_2 have been explored, including the electrochemical reduction of CO_2 (eCO_2RR). This method uses relatively mild reaction conditions and, most importantly, a catalyst, with which the CO_2 is reduced to several added-value products through the application of electrical energy [1]. Metal-Organic Frameworks (MOFs) are porous and crystalline 3D structures made of metal centres and organic ligands, that have shown to be effective catalysts for the eCO_2RR, yielding useful chemical compounds, such as hydrocarbons and alcohols [2]. This work is focused on the synthesis of several MOFs that contain different metal ions, including copper(II), iron(III), nickel(II), and zirconium(IV), and their electrocatalytic activity for CO_2 conversion. Both the direct (cathodic deposition) and indirect (electrophoretic deposition) electrosynthesis methods have been investigated to produce MOF coatings of Cu-MOF-74, Cu-ade, Fe-MIL-101 and NH_2-UiO-66 onto electrodes, and these were tested as electrocatalysts for the eCO_2RR using cyclic voltammetry and bulk electrolysis experiments.

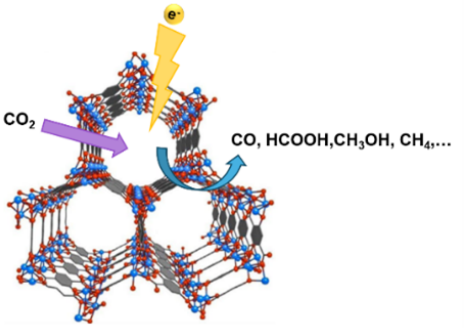


Figure 1: Scheme of the structure of a MOF (Cu-MOF-74) and several possible products of the eCO_2RR. Adapted from [3].

References

[1] Wiranarongkorn, K. et al. J. CO_2 Util. 2023, 71, 102477. [2] Li, L. et al. Chem. Soc. Rev. 2022, 51, 1234. [3] Sladekova, K. et al. Adsorption 2020, 26, 663-685.

Acknowledgements

CQE and IMS acknowledge financial support from FCT (Projects UIDB/00100/2020, UIDP/00100/2020, LA/P/0056/2020). SR thanks FCT for the contract 2020.02134.CEECIND. PNM thanks FCT and RSC for financial support (grants PTDCQUI-QIN0252$_2$021, $R21 - 7511142525)and FCT forthecontract CEECIND/00509/2017.$

OC3 - Identification of tissue specific dependencies between cancer driver gene mutations and their interactors abundances

Vital, M. F. S. (1,2); Miranda, J. A. I. (1,2); Carrolo, M. (3); Pinto, F. R. (1,2)

(1) - BioISI – Institute for Biosystems and Integrative Sciences, Faculty of Sciences, University of Lisbon, Lisbon, Portugal. (2) - Department of Chemistry and Biochemistry. (3) - Hematology and Oncology Department, CUF Oncologia, Lisbon, Portugal.

Cancer develops through the accumulation of mutations in driver genes. However, tissue-specific dependencies between drivers and their interactors at the protein level are poorly understood [1]. Our objective is to identify Driver-neighbour physical INTeractions (DINTs) associated with cancer development using a comparative interactome analysis across individuals and cancer types. We leverage data from The Cancer Genome Atlas Pan-Cancer cohort and protein-protein interaction networks (HuRI, Apid, BioGRID, STRING, and Omnipath). We analysed 2563 driver genes across 7712 individuals, quantifying the relationship between driver gene mutations and interactor expression profiles. Using Spearman correlation and regression models, we identified DINTs positively or negatively linked with driver mutation status, suggesting their involvement in cancer progression or suppression. To ensure these associations were not directly caused by driver mutations, we excluded interactors with similar gene expression changes between paired tumour and normal tissues from individuals with mutations in the interacting driver. Drivers were divided into classes according to frequencies of positive and negative DINTs. A functional enrichment analysis provided evidence of specific biological roles in each of these driver classes. Additionally, Kaplan-Meyer analysis identified significant impact of neighbours involved in multiple DINTs on patient overall survival. In summary, our work identified a set of DINTs potentially involved in cancer progression, contributing to a deeper understanding of tissue-specific driver gene effects and the identification of potential targets for cancer therapy.

References

[1] Ostroverkhova, D., et al. Trends Mol. Med. 2023, 29, 554–566.

Acknowledgements

Work supported by UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 (DOI: 10.54499/UIDP/04046/2020) Centre grants from FCT, Portugal (to BioISI).

OC4 - 5-amino-11H-indolo[3,2-c] isoquinolines: Small Molecules To Inhibit DHX36-G4 Interactions In C-MYC Promoter

Aljnadi, I.(1,2); Bahls, B. (1,2); Mendes, E. (2); Maçôas, E. (3); Victor, B. L. (2); Russell, R (4); Paulo, A . (1)

(1) - Medicinal Organic Chemistry Group, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal. (2) - BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal. (3) - CQE and IMS, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal. (4) - Department of Molecular Biosciences, University of Texas at Austin, Austin, Texas 78712, United States.

In recent years, researchers have focused on targeting G-quadruplex in DNA as an innovative approach for cancer therapy. G-quadruplex (G4) is a secondary structure formed by the folding of guanine-rich DNA sequences, where guanine moieties form two or more planar quartets linked together by Hoogsteen H-bonding. The biological roles of G4s were correlated with their prevalence within the promoter regions of oncogenes such as cMYC [1]. The formation of G4 in the c-MYC promoter decreases the oncogene transcription. Thus, cancer cells recruit helicases (e.g. DHX36) that unfold G4 (FIG1A.) restoring the c-MYC expression and cancer cells proliferation [1,2]. Targeting the unfolding process of G4 in c-MYC led by DHX36 represents a promising anticancer strategy. In this context, we explored the potential of indoloisoquinolines (IDiQs) as c-MYC G4-DHX36 interaction inhibitors. We initially docked a library of IDiQs derivatives with the crystallographic structure of c-MYC G4 to select the best side chains. We then synthesized a small library of IDiQs and evaluated the compounds' binding affinity to G4 by fluorescence titration (FIG1B.). All compounds showed a good binding affinity with Ka values of 106-107 M-1. Moreover, the primary assessment of the enzymatic inhibition activity of DHX36 revealed an inhibitory effect of some compounds that was consistent with the titration results. These results support our project design approach to target c-MYC G4 interaction with DHX36 and its unfolding activity.

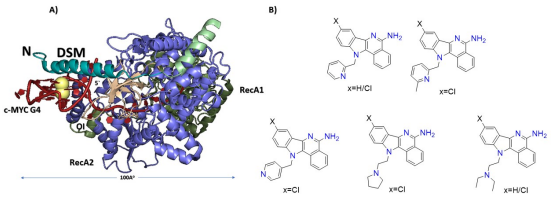


Figure 1: A): c-MYC G4 in complex with DHX36 (PDB 5VHE). B): Structure of synthesized indoloisoquinolines.

References

[1] Mendes, E. et al. Pharmaceuticals 2022, 15, 1–58. [2] Chen, M. C. et al. Nature 2018, 558, 465.

Acknowledgements

FCT to projects PTDC/QUI-QOR/29664/2017 (A. Paulo); PTDC/BIA-BFS/28419/2017 (B. L. Victor); UIDB/04046/2020UIDP/04046/2020 (BioISI) and UIDB/04138/2020-UIDP/04138/2020 (iMed). I. Aljnadi acknowledges Global Platform for Syrian Students, ULisboa, and FCT (2023.02872.BD) for a PhD scholarship.

OC5 - Tau phase separation is regulated by the calcium-binding S100B chaperone

Moreira, G.G. (1,2); Gomes, C.M. (1,2).

(1) - BioISI — Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal. (2) - Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

The phenomenon of liquid-liquid phase separation (LLPS) involving tau is increasingly acknowledged as a contributory process in the onset of tau aggregation and the generation of pathogenic conformers within Alzheimer's disease (AD). Neuroinflammation accompanies tau pathology, with late-stage astrocyte-released alarmin exacerbating the condition, while early inflammatory responses encompass protective functions. This applies to the Ca^{2+}-binding protein S100B, which we recently implicated as a proteostasis-regulator that inhibits amyloid-beta [1] and tau aggregation/seeding [2]. These findings suggest a broad holdase-type chaperone function for S100B in counteracting the malformation of protein structures. Our study aims to elucidate S100B's role in tau LLPS. We employed PEG-induced tau LLPS followed by light absorbance. Co-localization of S100B within tau droplets was achieved using fluorescence-labelled proteins. Evaluation of droplet fluidic characteristics encompassed FRAP and fusion events. Phase diagrams indicate significant suppression of tau droplet formation by Ca^{2+}-S100B, preserving droplet liquid properties. Introduction of Ca^{2+} to PEG-induced LLPS with apo-S100B promptly reduces tau droplet levels, highlighting the dynamic, calcium-triggered nature of Ca^{2+}-S100B's action. Also, S100B effectively halts PEG-free Zn^{2+}-induced tau LLPS due to its combined Zn^{2+}-buffering and tau-interaction. Our results establish S100B as a Ca^{2+}-dependent suppressor of tau LLPS. Collectively, these findings suggests that S100B, functioning as a chaperone, also regulates phase-separated systems, strengthening its pivotal role as a proteostasis regulator in early neurodegeneration.

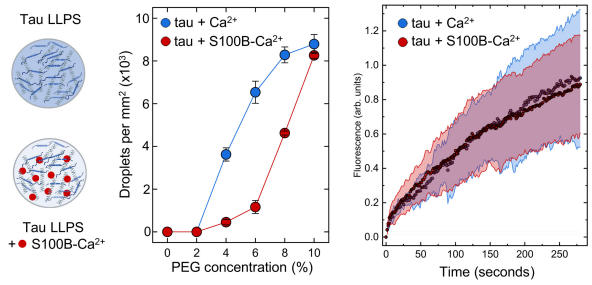


Figure 1: S100B-Ca^{2+} inhibits PEG-induced tau LLPS and colocalizes with tau droplets without compromising liquid-like droplet properties.

References

[1] Cristovão, J.S., et al. Sci. Adv. 2018, 4, eaaq1702. [2] Moreira, G.G., et al. Nat. Commun. 2021, 12, 6292.

Acknowledgements

Work funding by the European Union for funding Twinning Grant EU-TWIN2PIPSA/GA101079147, supported by UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 (DOI: 10.54499/UIDP/04046/2020) centre grants from FCT, Portugal (to BioISI), PhD grant (DOI: 10.54499/2020.06443.BD, to GGM) and Agilebio for funding LabCollector Scientific Award 2021 (to CMG).

OC6 - Synthesis and biological evaluation of novel guanidino and guanidinopurine-containing nucleosides

Szilagyi, J. (1); Nunes, R. (1), Silva, J. (2); Alves, C. (2); Martins, A. I. (2); Csuk, R. (3); Xavier, N. M. (1)

(1) - Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa. (2) - MARE-Marine and Environmental Sciences Centre/ARNET-Aquatic Research Network, Polytechnic University of Leiria. (3) - Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg.

Synthetic nucleosides, nucleotides, and their analogs/mimetics have garnered significant interest in both organic and medicinal chemistry due to their diverse biological properties. Several nucleoside and nucleotide analogs have found clinical application as anticancer or antiviral drugs by interfering with nucleic acid biosynthesis [1]. Moreover, these molecules have demonstrated antimicrobial effects [2] and inhibition of cholinesterases [3]. The ongoing quest for novel nucleos(t)ide analog structures that can offer innovative mechanisms of action and therapeutic opportunities remains a focal point of research.

In this communication, we present the synthesis and biological evaluation of novel 5-azido/guanidino nucleosides derived from a xylofuranose template. Additionally, we outline the synthetic route for the synthesis of novel guanidino-purine nucleoside derivatives, following a similar approach. Our synthetic strategy utilized diacetone-D-glucose as a precursor, involving the preparation of an acetylated 5-azidoglycosyl donor. This donor was then N-glycosylated with uracil or a purine derivative, leading to the formation of guanidine derivatives through a guanidinilization reaction. An intriguing result of the glycosyl donor precursor synthesis was the unexpected formation of an imino sugar via an intramolecular Boyer reaction.

We conducted antiproliferative assays on a panel of cancer cell lines and studied the compounds' ability to inhibit cholinesterases. Herein, we present and discuss our findings

References

[1] a) Jordheim, L. P et al. Rev. Drug. Discov. 2013, 12, 447-464; b) Shelton J et al. Chem. Rev. 2016, 116, 14379. [2] Serpi. M et al. J. Med. Chem. 2016, 59, 10343-10382. [3] a) Pereira R. G. et al. Eur. J. Org. Chem. 2018, 2667-2681; b) Batista. D et al. Pure Appl. Chem. 2016, 88, 363-379.

Acknowledgements

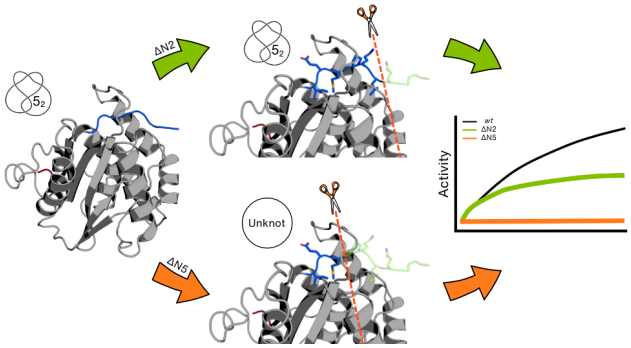
Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). This work was also supported by FCT through project EXPL/MED-QUI/1017/2021 (http://doi.org/10.54499/EXPL/MED-QUI/1017/2021) and grant CEECIND/03881/2018 (https://doi.org/10.54499/CEECIND/03881/2018/CP1553/CT0007).

OC7 - Untying the Knot: Unraveling the Functional Mysteries of Knotted Proteins via UCH-L1 Investigation

Ferreira, S. G. F. (1); Sriramoju, M. K. (3); Hsu, S.-T. D. (3); Patrícia F. N. Faísca (2); Miguel Machuqueiro (1)

(1) - BioISI, Departamento de Química e Bioquímica, FCUL, 1749-016 Lisboa, Portugal. (2) - BioISI, Departamento de Física, FCUL, 1749-016 Lisboa, Portugal. (3) - Institute of Biochemical Sciences, National Taiwan University, Taipei, 11529, Taiwan and International Institute for Sustainability with Knotted Chiral Meta Matter (WPI-SKCM2), Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan

Knotted proteins, distinguished by the presence of an open knot within their native conformation, gained attention following the advent of a knot detection method in 2000 [1]. Despite their complex folding kinetics, these proteins are evolutionarily conserved across all life kingdoms, fulfilling roles such as enhancing stability and resisting degradation [2]. However, a consensus on knots functional implications remains unclear. Hence, we focus on UCH-L1, a monomeric protein with a 5_2 knot, pivotal in the ubiquitin-dependent proteolytic pathway, and linked to neurodegenerative diseases. UCH-L1 acts as a cysteine protease, featuring a catalytic triad. In its apo structure, the triad is misaligned for catalysis, but upon ubiquitin binding, a conformational rearrangement occurs bringing the residues into closer proximity, enhancing enzymatic activity. The unanswered question concerning the function of the knot stands as the primary driving force behind this research. In order to seek an answer we conducted classical MD simulations alongside in vitro experiments to investigate the impact of the knot on the catalytic activity of UCH-L1. By engineering unknotted variants via N-terminus truncation, we discovered that removing the first two N-terminal residues causes partial loss of enzyme activity, while maintaining the secondary structure and knotted topology. In contrast, removing five N-terminal residues, which disrupts the native structure and topological state, results in complete loss of enzymatic activity. These results demonstrate the dependence of UCH-L1's catalytic activity on N-terminus integrity and overall structure, intricately linked to its knotted topology.



References

[1] Taylor, W. R. Nature 2000, 406, 916. [2] Sułkowska,J. I., et al. Proc. Natl. Acad. Sci. U. S. A. 2008, 105, 19714.

Acknowledgements

The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020 UIDP/04046/2020 and grants CEECIND/02300/2017 and UI/BD/153055/2022. This study was also supported by the European Union (TWIN2PIPSA - Twinning for Excellence in Biophysics of Protein Interactions and Self-Assembly, GA 101079147).

OC8 - Oxidative Desulfurization of Fuels Catalysed by $MoO_3 - Fe_3O_4$ Nanoparticles

Henriques, A. C. (1); Moreira, P. (1); Nunes, C. D. (1)

(1) - CQE, Faculty of Sciences, University of Lisbon, Portugal.

Air pollution has always been a problem due to its consequences on human health and the environment. The combustion of fossil fuels is its main source because it releases sulfur oxides from compounds present in the fuel, such as thiophene and derivatives, which mainly cause acid rains, cardiovascular and asthmatic diseases, and cancer. It is imperative to remove these compounds. One way to do that is to submit the fuels to a green process known as oxidative desulfurization (ODS) using heterogeneous catalysts to promote the reaction [1]. The present work aimed the use of MoO_3 anchored to Fe_3O_4 magnetic nanoparticles as catalyst to the ODS of methyl phenyl sulfide (MFS), diphenyl sulfide (DFS), dibenzothiophene (DBT) and 1 benzothiophene (1BT), using TBHP or H_2O_2 as oxidants and CH_3CN as solvent. The obtained results turned out to be very promising, yielding sulfoxides and sulfones which are often used in pharmaceuticals, organic chemistry, agrochemicals, etc. The catalyst selectivity was very high for sulfone in most cases. These results are very promising since sulfones can be used in various applications. A catalytic test with a mixture of all 4 substrates was also successfully conducted and showed very high conversions, opening the door to study this system in real fuel samples.

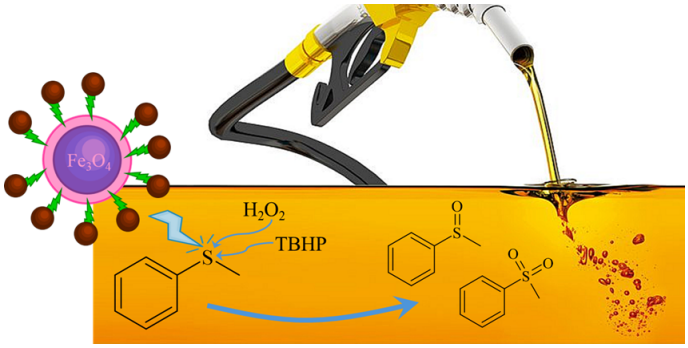


Figure 1: Illustrative scheme of the oxidative desulfurization of MFS catalyzed by $MoO_3 - Fe_3O_4$ nanoparticles

References

[1] Hossain, M. N. et al. Catalysts 2019, 9, 229.

Acknowledgements

This work was supported by Fundação para a Ciência e Tecnologia (FCT) through project with DOI 10.54499/2022.03236.PTDC, Centro de Química Estrutural, by UIDB/00100/2020 and UIDP/00100/2020 and Institute of Molecular Sciences an Associate Laboratory funded by FCT through project LA/P/0056/2020.

Flash Pitches

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

F1 - Identification and characterization of a novel library of single-domain antibodies which compete for RAGE binding and modulate S100B neurotrophic activity

Simões, M.C. (1,2); Cristóvão, J.S. (1,2); Pardon, E. (3); Steyaert, J. (3); Fritz, G. (4); Gomes, C.M. (1,2)

(1) - BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Portugal. (2) - Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal. (3) - VIB-VUB Center for Structural Biology, Brussels, Belgium. (4) - Institute of Biology, Department of Cellular Microbiology, University of Hohenheim, Stuttgart, 70599, Germany.

S100B is a multifunctional protein associated with intra- and extracellular roles, primarily found in the brain, where it plays crucial roles in cell proliferation, differentiation, and cell survival [1]. Depending on S100B concentration, this protein can have neurotrophic and neurotoxic activity, both which are receptor for advanced glycation end products (RAGE)-mediated [2]. The neurotoxic activity of S100B triggers proinflammatory responses exacerbating conditions such as traumatic brain injury and neurodegeneration [2]. Here, we report the discovery and characterization of nanobodies (VHHs) targeting dimeric and tetrameric S100B, which are the two most abundant oligomeric functional forms of the protein with the goal of modulating S100B-mediated RAGE activation. The selected VHHs recognized structural epitopes present in both S100B conformers but not in other S100 proteins. Two of the selected VHHs bind tetrameric S100B with high affinity, as determine by biolayer interferometry analysis and stable complex formation. Structural and docking analysis revealed preferential interaction sites of the VHHs on S100B implicated in RAGE interaction. In accordance, VHHs modulated RAGE-mediated neurotrophic activity of S100B in SH-SY5Y cells by inhibiting this activity. To specify which RAGE-domains are affected by VHHs binding to S100B, we performed competition binding assays and identified VHHs that selectively inhibit S100B engagement with specific RAGE-domains. These finding uncover VHHs as powerful investigational tools to elucidate molecular and cellular mechanisms through the modulation of RAGE-mediated S100B functions and inspire potential therapeutic applications.

References

[1] Donato, R., et al. Biochim. Biophys. Acta. 2009,1793, 1008-22. [2] Bresnick, A.R., et al. Nat. Rev. Cancer 2015,15, 96-109.

Acknowledgements

EU for funding Twinning Grant EU-TWIN2PIPSA/GA101079147, FCT/MCTES (Portugal) for funding UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/MULTI/04046/2020 (DOI: 10.54499/UIDP/04046/2020) (BioISI) and FCT-Portugal PhD grant to MCS (2022.11023.BD).

F2 - Ruthenium-peptide conjugates as potential new breast cancer therapy agents

Marques, B. (1); Nogueira, J. M. F. (1); Morais, T. S. (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

Breast cancer is the most diagnosed and second deadliest type of cancer among women worldwide, affecting 2.26 million women only in 2020. Metastases are the major cause of death by cancer, as it renders the disease incurable due to the lack of efficient and selective treatment for metastatic cancers. Moreover, late diagnosis of cancer prohibits metastasis prevention and greatly reduces the patient's chance of survival [1]. Therefore, a novel therapeutic solution is urgently needed. Metastatic breast cancer cells frequently overexpress fibroblast growth factor receptors (FGFR), which can be exploited as a target for the delivery of anticancer agents. This strategy has been adopted by T.S. Morais [2], for the development of novel smart ruthenium metallodrug delivery systems (SMDS) that comprise a cancer-targeting peptide that recognizes FGFR with high affinity, tethered to a known cytotoxic Ru(II)-cyclopentadienyl complex through a linker responsive to the acidic tumoral microenvironment. Herein we report the synthesis of a new FGFR-targeting SMDS by conjugating a peptide to a Ru(II)-cyclopentadienyl complex through two different pH-sensitive linkers (Figure 1). The new compounds were characterized by high performance liquid chromatography (HPLC) and electrospray mass spectrometry (ESI-MS). The release behavior of the oxime linker in aqueous solution was studied over time by HPLC, at pH values that mimic the tumor microenvironment (6.8) and the healthy tissues/bloodstream (7.4).

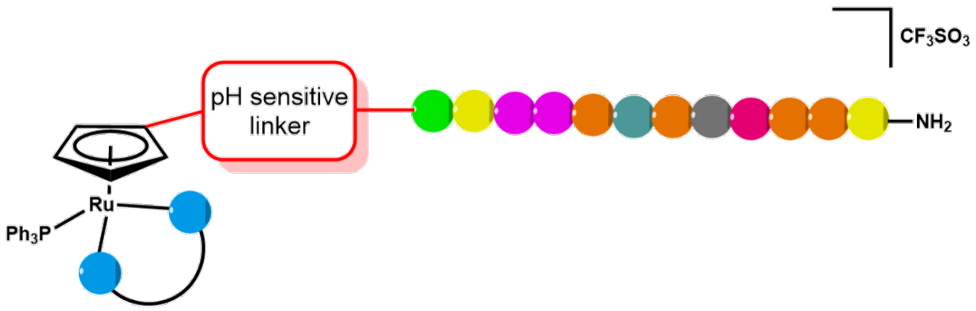


Figure 1: Figure 1. Schematic structure of the newly synthesized SMDS.

References

[1] Chhikara, B. et al. Chem. Biol. Lett. 2023, 10, 451. [2] Machado, J. et al. Dalton Trans. 2024, 53, 7682-7693.

Acknowledgements

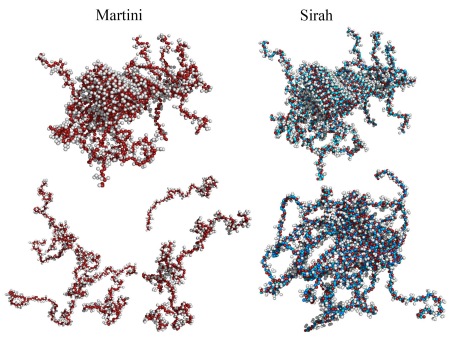
This work was funded by Fundação para a Ciência e Tecnologia (FCT), I.P./MCTES through the projects PTDC/QUI-QIN/0146/2020 (Arrows2cancer, DOI 10.54499/PTDC/QUI-QIN/0146/2020), UIDB/00100/2020 (CQE, DOI 10.54499/UIDB/00100/2020), LA/P/0056/2020 (IMS, DOI 10.54499/LA/P/0056/2020). FCT, POPH and FSE- European Social Funds are acknowledged for the Individual Call to Scientific Employment Stimulus 2022.00028.CEECIND/CP1722/CT0005 (DOI 10.54499/2022.00028.CEECIND/CP1722/CT0005) project.

F3 - Coarse-Grained and All-Atom Molecular Dynamics Study of the Structure, Dynamics, and Aggregation of α-Synuclein

Martins, G. (1); Galamba, N. (1)

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

Intrinsically disordered proteins (IDPs) have a wide range of conformations that are challenging to study experimentally. Molecular dynamics (MD) can explore these conformations but is limited by sampling and force field (FF) accuracy. α-synuclein (α-syn) is a 140-amino acid IDP implicated in Parkinson's disease (PD), and understanding its structure and aggregation is key to design potential aggregation inhibitors for PD. Coarse-grained (CG) models can overcome sampling limitations, though they lose atomic detail. While CG models can describe individual monomer structures, their ability to describe aggregation is less understood. Herein, we explore the structure and dynamics of α-syn using two CG (Martini3 and Sirah2) and two all-atom FFs (Amber99sb and Charmm36m). We assess protein-water interactions and the need for enhanced sampling to adequately sample conformations, and the binding free energy, through umbrella sampling, of CG models of α-syn and CG/all-atom models of an 11-mer peptide (NACore), from the α-syn's NAC region. Our findings reveal significant disparities in aggregation despite the structural similarities observed in Martini3 and Sirah2 models of α-syn, with augmented protein-water interactions. The Martini3 fibril lacks stability, and the binding free energy of α-syn and NACore is positive, unlike the Sirah2 model. Sirah2 shows excessively strong terminal interactions, leading to end-to-end orientation in zwitterionic peptides. Our results suggest that Sirah2 is promising for studying protein-protein and protein-drug aggregation processes.



References

Acknowledgements

Gabriel Martins acknowledges financial support from Fundação para a Ciência e a Tecnologia (FCT) of Portugal for a PhD scholarship (2021.05348.BD). Nuno Galamba acknowledges financial support from FCT (CEECIND/00821/2017). Gabriel Martins and Nuno Galamba acknowledge support from UIDB/04046/2020 (doi: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 center grants from FCT, Portugal (to BioISI) and from the 2022.15913.CPCA.A1, the 2022.15882.CPCA.A2, and the 2023.12001.CPCA.A1 grants of the Portuguese National Distributed Computing Infrastructure (http://www.incd.pt).

F4 - Solventless synthesis of iminophosphine ligands and their coordination to Ru(II) and Cu(I) metals

Mendes, I. S. V. (1); Gomes, C. S. B.(1); Rosa, V. (1)

(1) - LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology (NOVA FCT), NOVA University Lisbon, 2829-516 Caparica, Portugal.

The development of green synthetic methodologies is paramount in modern chemistry in order to reduce their environmental footprint. The utilization of emerging techniques such as mechanochemistry, microwaves irradiation or sonication are good examples of new methods that can lower the energetic needs of reactions and decrease the amount of side products. The use of solvents in organic chemistry is often responsible of more than 90% of the weight of the total reaction mass, so reducing or eliminating its use, is paramount to lower its environmental impact [1]. Reactions using one of the reactants as solvent, so called neat reaction conditions, are one of the most effective ways to eliminate the use of solvent medium. Following this procedure, we used it on a Schiff base reaction. We employed an aldehyde, (diphenyl)phosphine benzaldehyde, and reacted it with various liquid amines (anilines and primary alkyl amines) [2]. The ability of the obtained compounds to coordinate to metals centres and to be used as catalysts was also assessed. Three of such compounds were obtained, using copper(I) iodide, tetrakis-acetonitrile copper(I) tetrafluoroborate and ruthenium(II) chloride cyclooctadiene. All the complexes were fully characterized by spectroscopic techniques as well as single crystal X-ray diffraction. The copper complexes were tested as catalysts for the 1,3 dipolar cycloaddition of azides and alkynes.

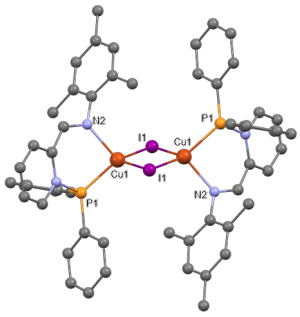


Figure 1: Mercury diagram of the dimeric copper iodide complex

References

[1]Sheldon, R. A. Green Chem. 2017, vol. 19, no. 1, pp. 18–43. [2]Vasilenko, V. et al. Beilstein J. Organic Chem. 2016, vol. 12, pp. 846–853.

Acknowledgements

This work was supported by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds.

F5 - Striving for new Bioadhesives inspired by Sea Urchin proteins

Santos, M. R. (1); Gomes, C. M. (1); Santos, R. (2); Henriques, B. J. (1)

(1) - BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal. (2) - MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Nowadays there is a great need for biological adhesives that are non-cytotoxic and efficient in wet/humid environments for biomedicine and biotechnology applications (for example, to be used in surgical adhesives). It is known that marine invertebrates produce secretions with remarkable adhesive properties in the presence of seawater (similar to physiological fluids in its high dielectric and ionic strength), which can inspire the development of new biomimetic adhesives. We have been studying sea urchins' adhesives, having identified Nectin from Paracentrotus lividus as an important adhesive protein present in its adhesive organs (tube feet) and adhesive secretions [1]. Nectin has six galactose-binding discoidin-like (DS) domains, which are thought to be important for its adhesive function [1-3]. Aiming to develop a new bioadhesive inspired in sea urchins, we are currently developing a project focused on the study of constructs whose design is based on the Nectin protein sequence. We have successfully produced one construct, that we are now in the process of characterising, in respect to structure, conformational stability, aggregation propensity, and adhesive strength, using techniques like circular dichroism (CD), fluorescence, TEM and surface coating assays. Interestingly, in the presence of certain salts at high concentration, like seawater conditions, the construct appears to aggregate as indicated by Thioflavin-T fluorescence assays and TEM imaging, evidencing possible fibre formation. Moreover, preliminary data of surface coating assays indicates that the construct adsorbs to glass surfaces forming an heterogenous coating.

References

[1] Santos, R. et al. J Proteomics. 2013, 79, 100-113. [2] Pjeta, R. et al. Int J Mol Sci. 2020, 21, 946. [3] Costa, C. et al. Mol Divers. 2010, 14, 653-665.

Acknowledgements

This research was supported by FCT/MCTES, Portugal, through the strategic project DOI:10.54499/UIDB/04292/2020, DOI:10.54499/UIDP/04292/2020 (MARE), DOI:10.54499/LA/P/0069/2020 (Associate Laboratory ARNET), DOI:10.54499/UIDB/04046/2020 (BioISI), DOI:10.54499/CEECINST/00032/2018/CP1523/CT0006 (R.S.), DOI:10.54499/2021.02218.CEECIND/CP1650/CT0008 (B.J.H.). This research is funded by the European Union (TWIN2PIPSA, GA 101079147). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

F6 - APIs calorimetric studies in deep eutectic solvents

Chorão, M. (1,2); Nunes, N. (1,3); Elvas-Leitão, R. (1,3); Martins, F. (1,2)

(1) - Centro de Química Estrutural – Institute of Molecular Sciences, Universidade de Lisboa, Portugal. (2) - Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal (3) - Departamento de Engenharia Química, Instituto Superior de Engenharia de Lisboa, IPL, Portugal

Deep eutectic solvents (DES) are seen as alternative media to organic solvents and as an option in the pharmaceutical industry to enhance the solubility of active pharmaceutical ingredients (APIs). DES are composed of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) and are defined as a mixture of two or more components, that can be solid or liquid, that, at a particular composition, have a melting point lower than that of its pure components [1]. In this work, the solubility of different APIs, namely acetaminophen, acetylsalicylic acid, ibuprofen, isoniazid and salicylic acid was tested in different choline chloride based DES. Solution enthalpies for the systems where the APIs were found to be soluble, i.e., choline chloride:1,2-propanediol (1:3) and choline chloride:urea:1,2-propanediol (1:1:2) were measured at 298.15 K and at infinite dilution using a Thermometric precision solution calorimeter and compared with those in pure organic solvents. The DES water content was monitored with a hybrid KEM Kyoto Electronics Karl-Fischer moisture titrator and was always less than 1%. For characterization purposes, the Kamlet-Taft solvatochromic parameters, α, β, and π^* and the Dirmroth-Reichardt polarity parameter, ETN, as well as the refractive indices of the two DES were also determined. Solvatochromic parameters were very similar for both eutectic mixtures, whereas refractive indices obtained for $ChCl : Urea : 1,2 - Pr(OH)_2$ were higher than those for $ChCl : 1,2 - Pr(OH)_2$. A comparison with other choline chloride based DES involving ethylene glycol and glycerol was also attempted.

References

[1] Dias, M, et al. J. Sol. Chem. 2022, 51 (5), 577-593.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

F7 - Investigation of the glycerol-3-phosphate: quinone oxidoreductases from Staphylococcus aureus and Pseudomonas aeruginosa

Pires, P. (1); Pereira, M. (1)

(1) - University of Lisbon, Faculty of Sciences, Department of Chemistry and Biochemistry, and BioISI – Biosystems Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

Staphylococcus aureus and Pseudomonas aeruginosa are opportunistic pathogens, and have become major public health concerns because of the increased incidence of their drug resistance. The two organisms are responsible for both hospital and community-acquired infections. S. aureus and P. aeruginosa are both facultative anaerobes that present a great ability to adapt to diverse environmental conditions, especially during host colonization, which makes them exceptional opportunistic pathogens. Their adaptability comes from their metabolic versatility, which in part is due to the vast array of quinone reductases that connect different metabolic pathways to the respiratory chain [1]. Importantly the two bacteria produce quinones with different structures, S. aureus synthesizes menaquinone while P. aeruginosa produces ubiquinone. Most relevant, P. aeruginosa also produces 2-Heptyl-4-hydroxyquinoline-N-oxide (HQNO), a quinone analogue that works as an inhibitor of most quinone reductases and which confers to this bacterium a competitive advantage when colonizing the same niches with other bacteria. Glycerol-3-phosphate: quinone oxidoreductases (G3PQOs) are flavin-containing monotopic enzymes catalyze the oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate and concomitant reduction of quinone to quinol. We performed a comparative analysis of the G3PQOs from S. aureus and from P. aeruginosa. The production and purification of G3PQOs was achieved and successful initial biochemical characterization of G3PQOs were performed. We observed that the enzyme from S. aureus is inhibited by HQNO, whereas that from P. aeruginosa is insensitive to this inhibitor.

References

[1] Tong, S. et al. Clin. Microbiol. Rev. 2015, vol. 28, 603–661.

Acknowledgements

PMP is recipient of a fellowship by Fundação para a Ciência e a Tecnologia (2023.03000.BD). The work was funded by Fundação para a Ciência e a Tecnologia (PTDC/BIA-BQM/2599/2021, DOI: 10.54499/PTDC/BIA-BQM/2599/2021 to MMP). The project was further Work supported by UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 (DOI: 10.54499/UIDP/04046/2020) Centre grants from FCT, Portugal (to BioISI).

F8 - Pharmacokinetic profiling of Ruthenium and Selenium hit compounds: guiding the optimization of alternative therapeutic options to overcome multidrug resistance in cancer treatment

Henriques, C. (1,2); Baptista, D. B. (1); Teixeira, R. G. (2); Valente, A. (2); Antunes, A. M. M. (1)

(1) - Centro de Química Estrutural, Institute of Molecular Sciences, Department of Chemical Engineering, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal; (2) - Centro de Química Estrutural, Institute of Molecular Sciences and Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

Cancer is the second leading cause of death and illness in Europe, with multidrug resistance (MDR) complicating treatment efforts. Our research focuses on developing ruthenium complexes, specifically $[Ru(\eta 5 - C_5H_4R)(4,4\prime\text{-R}\prime\text{-2,2}\prime\text{-bipy}))(PPh_3)][CF_3SO_3]$ (R = CHO or CH_2OH; R' = dimethoxy or dimethyl). These compounds show substantial efficacy against resistant non-small cell lung cancer cells, exhibiting collateral selectivity by targeting only resistant cells. This selective cytotoxicity is due to their inhibition of P-gp and MRP1 efflux pumps, which are proteins responsible for MDR [1,2]. Additionally, selenium-containing chrysin (SeChry) has shown promise in overcoming MDR, particularly in combating cisplatin resistance across various cell lines. This efficacy is attributed to its glutathione peroxidase (GPx)-like activity and its inhibitory effects on thioredoxin reductase (TrxR) and cystathionine β-synthase (CBS) [3,4]. These preliminary findings suggest that a combinatorial therapy with selenium and ruthenium-based compounds could be a promising approach for addressing the challenge of MDR in cancer treatment. However, a deep understanding of their metabolic fate is crucial to identify structural vulnerabilities and guide optimization efforts, preventing late-stage drug attrition due to toxicity [5]. Our study evaluates their in vitro stability against plasma and liver metabolizing enzymes, and their metabolic profile in human liver microsome incubations using liquid chromatography coupled with tandem high-resolution mass spectrometry (LC-HRMS/MS). This strategy allowed the identification of structural liabilities, guiding strategies for the hit to lead optimization.

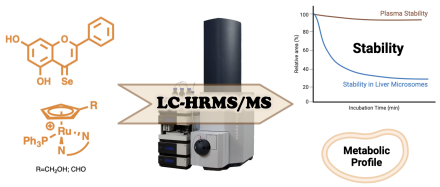


Figure 1: Scheme 1: Pharmacokinetic profiling of Ruthenium and Selenium Complexes.

References

[1] Teixeira, R. G. et al. Inorg Chem Front. 2021, 8, 1983–1996. [2] Teixeira, R. G. et al. J Med Chem. 2023, 60, 14080-14094. [3] Martins, I. L. et al. J Med Chem. 2015, 58, 4250-4265. [4] Santos, I. et al. Nutrients. 2019, 11, 2523. [5] Kalgutkar, A. S. J Med Chem. 2020, 63, 6276–6302.

Acknowledgements

Fundação para a Ciência e a Tecnologia (FCT) is acknowledged for funding Centro de Química Estrutural (UIDB/00100/2020 and UIDP/00100/2020) and Institute of Molecular Sciences is an Associate Laboratory (LA/P/0056/2020) and project 2022.06292.PTDC. Joint funding from FCT and the COMPETE Program through grant RNEM-LISBOA-01-0145-FEDER-022125 funding are also gratefully acknowledged. Catarina Henriques and Andreia Valente acknowledge FCT for their PhD fellowship (UI/BD/154408/2023) and CEECIND 2017 (CEECIND/01974/2017), respectively.

F9 - Inhibition of GRK5 with CCG-273441 as a potential novel strategy to restore p.Phe508del-CFTR traffic

Caleiro, M. F. (1); Railean, V. (1); Amaral, M. D. (1); Botelho, H. M. (1)

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

Cystic Fibrosis (CF) is the life-limiting autosomal recessive disease that affects more individuals among the Caucasian population. It is caused by variants in the CFTR gene, which encodes a chloride and bicarbonate channel expressed at the apical plasma membrane (PM) of epithelial tissues such as the airways. The most common pathogenic CFTR gene variant – p.Phe508del, 85% of CF cases – leads to CFTR protein misfolding, ER retention, premature degradation and, ultimately, impairment in PM expression and transepithelial anionic transport. Our group recently identified GRK5 as a novel p.Phe508del-CFTR regulator: GRK5 inhibition using genetic (siRNA) or pharmacological tools partially restores p.Phe508del-CFTR trafficking to the PM in CF cellular models. This restoration was additive to CFTR modulator drugs, which partially overcome the molecular defects of selected CFTR variants [1,2]. In this work, we assessed the rescue of p.Phe508del-CFTR traffic and function by a new GRK5 inhibitor, CCG-273441 [3]. GRK5 was inhibited in bronchial epithelial cell lines (CFBE and 16HBE) with CCG-273441, both alone or in combination with CFTR modulators, and its effect on the regulation of p.Phe508del-CFTR traffic was assessed through biochemical (post-ER processing by Western blot) and functional methods (quantification of CFTR activity using the Ussing Chamber). Our results suggest that CCG-273441 is additive to the CFTR modulators Tezacaftor and Elexacaftor, suggesting a potential strategy to improve CF management. We will discuss the biomedical relevance of the GRK5 regulator and how we are identifying the genes which transduce GRK5 inhibition into CFTR traffic regulation.

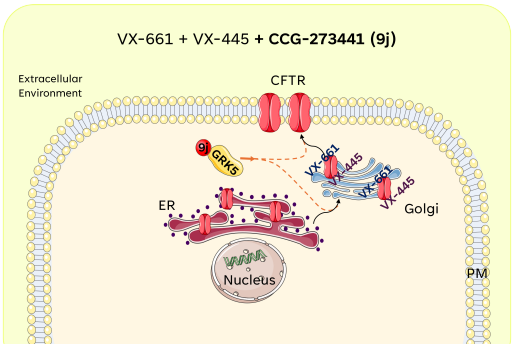


Figure 1: Fig 1. CCG-273441 effect on the rescue of p.Phe508del-CFTR traffic in combination with CFTR modulators.

References

[1] Botelho, H. M. et al. (2024, under revision). [2] Patent PCT/IB2023/051813. [3] Rowlands, R. A. et al. J Med Chem. 2021, 64, 566-585.

Acknowledgements

Work supported by centre grants UIDB/04046/2020 and UIDP/04046/2020 (to BioISI) and grant NewKinCF (DOI: 10.54499/2022.03453.PTDC, to HMB), from FCT/MCTES. MFC was a recipient of a BioISI Junior BII fellowship.

F10 - The Multifacety of MOFs: From Structural Determination to Photocatalytic Hydrogen Production

Lúcio, M. (1); Rosa, V. (1); Gago, S. (1); Gomes, C. S. B. (1)

(1) - LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal.

Structural characterization of non-crystallizable compounds is an impending issue needing to be solved. Using single-crystal X-ray diffraction (SCXRD), the absolute configuration of structures can be determined, making it a very important characterization technique – but obtaining single crystals is not always an easy procedure. In 2013, Fujita and co-workers tried to overcome those challenges through the development of the Crystalline Sponge Method (CSM) for SCXRD analysis, which requires the encapsulation of guest molecules into the pores of a crystalline metal-organic framework (MOF). Fujita synthesized a versatile Zn-MOF for this purpose, but besides effective, it presents its own limitations, such as pore size and stability in aqueous media, hindering its applicability. Gaseous hydrogen is a promising candidate as a green and sustainable alternative energy source for the hydrocarbon systems currently employed. Moreover, MOFs are a popular material due to their large pores and high-specific surface area, hence they have been appointed as potential photocatalysts for hydrogen production. Herein, we report the development of Zr- and Zn-MOFs for their application as crystalline sponges and as photocatalysts for hydrogen production through water splitting. The materials were characterized by diffuse reflectance spectroscopy (DRS), infrared (IR), and, when possible, by powder and single-crystal X-ray diffraction (PXRD and SCXRD, respectively).

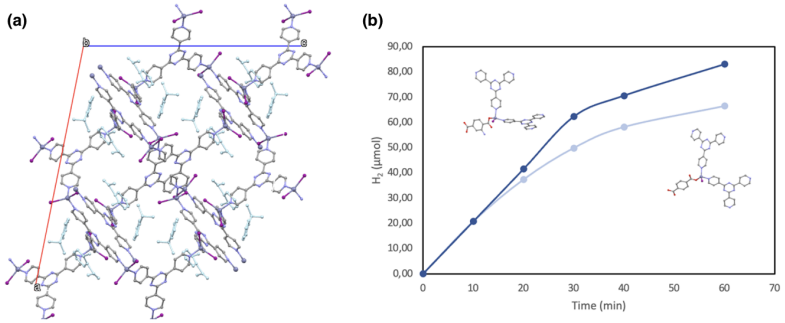


Figure 1: (a) Structural determination of o-Isopropylaniline by CSM and (b) Hydrogen production by Zn-MOFs through water splitting, using Na_2S/Na_2SO_3 as a sacrificial agent, irradiatated with a 300 W Xe lamp.

References

[1] Inokuma, Y. et al. Nature 2013, 495, 461. [2] Qian, Y et al. Adv. Funct. Mater. 2021, 31, 2104231.

Acknowledgements

This work was supported by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds.

F11 - The role of post-translational modifications in the dimerization of alpha and beta splicing isoforms of STAT3

Pimenta, C. (1); Murtinheira, F. (1); S. Rodrigues, M. (1); Herrera, F. (1)

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

STAT3 plays a key role in development, immune response, and cancer. STAT3 has 4 splicing isoforms, with STAT3α and STAT3β being the most abundant, which can form heterodimers. While the canonical functions of STAT3 are clearly mediated by Y705 phosphorylation, the impact of more than 80 possible post-translational modifications (PTMs) is unclear. The main goal of this work is to elucidate the putative role of PTMs on the heterodimerization, subcellular localization and transcriptional activity of STAT3α and STAT3β isoforms. To address this, we designed molecular tools based on BiFC to analyse the dynamics of STAT3α-STAT3α and STAT3α-STAT3β heterodimers, including plasmids with mutations in residues preventing key PTMs (Y705F and K685R). The response of STAT3 heterodimers to TNF-α and LIF cytokines was tested in HeLa STAT3-/- cells. STAT3α-STAT3β dimers present a nuclear-cytoplasmic distribution and accumulate in the nucleus in the presence of LIF, but not TNF-α. STAT3α homodimers with asymmetric PTM-resistant mutations at K685, K49 and S727 can accumulate in the nucleus in response to LIF. STAT3α-STAT3β heterodimers with PTM-resistant mutations at K685 are also able to accumulate in the nucleus in response to LIF. These results enhance our understanding of the role of STAT3 on fundamental cellular processes relevant to development, cancer, neurodegeneration, and inflammation.

References

[1] Diallo, M., et al., FEBS J. 2022, 289, 6235-6255. [2] Diallo, M„ et al., Cell. Onc. 2023.

Acknowledgements

CP was supported by MSc fellowship from BioISI.BioISI/FCUL Microscopy Facility, a node of the Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122). FH and MR were supported by centre grants UIDB/04046/2020 and UID/MULTI/04046/2020 (to BioISI) funded by FEDER funds through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and national funds through FCT (Ref. PTDC/FIS-MAC/2741/2021). FM was supported by a PhD fellowship from FCT (SFRH/BD/133220/2017). This project was also supported by a grant from the European Union (TWIN2PIPSA - Twinning for Excellence in Biophysics of Protein Interactions Self-Assembly, GA101079147)

F12 - Visible Light Photocatalytic Carboxylation of Carbonyl Compounds with CO_2

Hašková, L. (1,2); Realista S. (2); Martinho P. (2); Vyskocilova E. (1)

(1) - University of Chemistry and Technology - Department of Organic Technology, Prague, Czech Republic. (2) - Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa.

In recent years, a significant focus in organic chemistry has been the development of strategies utilizing carbon dioxide (CO_2). Carbon dioxide has emerged as a desirable C1 feedstock in organic synthesis due to its abundance and eco-friendly nature [1]. However, despite existing methods for carbonyl compound functionalization, carboxylation methods using CO_2 remain limited [2,3]. In this study, we investigate an alternative approach to carboxylation of carbonyl compounds with CO_2 under visible light irradiation. This strategy offers a sustainable and efficient route to valuable α-hydroxycarboxylic acids. Metal complexes of ruthenium and rhenium (Rubipy, Rebp, Rebpp) were prepared as photocatalysts, alongside synthesizing the sacrificial donor BIH. Benzaldehyde and benzophenone were chosen as model molecules for the carboxylation reactions. These reactions were conducted under various conditions, including blue LED light, solar simulator setups, and even natural sunlight, demonstrating the versatility of the approach. The synthesis of mandelic acid from benzaldehyde resulted in a 50% yield, limited by the formation of hydrobenzoin as a side product.

References

[1] Yan, S.-S et al. Chem. Sci. 2018, 9, 4873-4878. [2] Cao, G.-M. et al. Nat. Commun. 2021, 12, 3306. [3] Okumura, S. et al. Org. Lett. 2021, 23, 7194-7198.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). We are grateful to Fundação da Ciência e a Tecnologia, FCT, for Project PTDC/QUI-QIN/0252/2021. P.N.M. (CEECIND/00509/2017) acknowledge FTC for financial support.

Posters

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

P1 - Targeting Amyloid Deposits with Radiation Therapy: A New Frontier in Neurodegenerative Disease Management

Coelho, C. M. (1,2,3); Teubig, P. (1,2); Murtinheira, F. (1,3); Santos, P. (4); Mendes, F. (4); Reis, P. (5); Prudêncio, L. (5); Cortés-Llanos, B. (6); Galaviz, D. (1,2); Herrera, F. (1,3)

(1) - Faculdade de Ciências, Universidade de Lisboa (FCUL). (2) - Laboratório de Instrumentação e Física Experimental de Partículas (LIP). (3) - Instituto de Biosistemas e Ciências Integrativas (BioISI), FCUL. (4) - Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico (IST). (5) - Unidade Local de Saúde de Santa Maria (ULSSM), Centro Hospitalar Universitário Lisboa Norte (CHULN). (6) - Centro de Micro-Análisis de Materiales (CMAM), Universidad Autónoma de Madrid (UAM).

Amyloid deposits, resulting from abnormal protein accumulation, play a crucial role in causing organ dysfunction and degeneration. Radiotherapy (RT), a mainstay in cancer treatment for over 50% of patients, has the potential to extend beyond oncology and address extra-cranial amyloidosis. Furthermore, RT shows promise in treating amyloid-associated neurodegenerative disorders such as Alzheimer's, and Huntington's diseases [1]. Proton Therapy (PT) offers significant clinical advantages over conventional RT by minimizing damage to healthy tissues [2]. Our research investigates the potential of various RT modalities to combat toxic amyloid proteins involved in neurodegenerative disorders. Initial gamma-irradiation experiments conducted at C2TN demonstrated a dose-dependent reduction in pathological protein levels. Similar outcomes were observed with photon and electron irradiation experiments performed at ULSSM. PT experiments will be conducted at CMAM supported by dosimetry measurements. Monte Carlo simulations using TOPAS will be employed to model radiation effects on protein deposits. Additionally, an in vitro model is under development to study the impact of radiation on protein aggregation, using Thioflavin T as a probe. Our presentation will showcase the results from gamma-irradiation and current photon and electron experiments. We aim to broaden the applications of PT, enhancing its scope and potentially altering the progression of neurodegenerative disorders.

References

[1] Coelho, C.M., et al. Int. J. Mol. Sci. 2022, 23, 12265. [2] Paganetti, H., et al. Phys. Med. Biol. 2021, 66, 05RM01.

Acknowledgements

ProtoTera Grant - PRT/BD/151545/2021, Centre grants to LIP UIDB/50007/2020, UIDP/50007/2020 and LA/P/0016/2020 and UID/Multi/04349/2019 Centre grant to C2TN. Centre grants to BioISI UIDB/04046/2020 and UIDP/04046/2020 and the TWIN2PIPSA twinning grant from the European Research Council (ID: 101079147).

P2 - Converting the problematic carbon dioxide into added-value compounds using enzymes

Pereira, L. (1); Amador, A. (1); Bertinelli, A. (1); Moura I. (1), Moura, J.J.G. (1); Maia, L.B. (1)

(1) - LAQV, REQUIMTE, NOVA School of Science and Technology | NOVA FCT, Portugal

The atmospheric levels of CO_2 are causing huge and unpredictable impacts on Earth's climate due to its significant greenhouse effect. To stop the catastrophic consequences caused by climate change,CO_2 emissions must be greatly reduced. Additionally, it is necessary to develop new and more effective ways to convert this compound into added-value products [1,2]. In this context, the conversion of CO_2 into formate offers significant benefits for carbon recycling, since formate can be easily stored, transported and converted into various products highly interesting for the energy and chemical industry. To achieve the CO_2 conversion into formate, enzymes offer significant advantages,namely the selectivity and specificity of substrate and product, as well as the ability to run reactions at room temperature and pressure, in water, at neutral pH. Our main goal is to exploit formate dehydrogenase (FDH) [3] enzymes, that are able to catalyse the reduction of CO_2 to formate, to develop photochemical and electrochemical devices for the conversion of CO_2 into added-value compounds. Presently, we are characterising different FDH from sulfate reducing bacteria of the Desulfovibrio genus in order to identify the best CO_2 reducers. In this communication, our recent results on these studies will be discussed.

References

[1] Maia, L., et al. Carbon Dioxide Utilisation -The Formate Route 2021, 29-81. [2] Maia, L., et al. Inorg. Chim. Acta 2017, 455, 350-363. [3] Maia, L, et al. RSC, 2017, 1-80.

Acknowledgements

This work was supported by the PTDC/BTA-BTA/0935/2020 project and also by the Associate Laboratory for Green Chemistry - LAQV (UIDB/50006/2020 (DOI: 10.54499/UIDB/50006/2020) and UIDP/50006/2020 (DOI: 10.54499/UIDP/50006/2020)), which are financed by national funds from Fundacão para a Ciência e a Tecnologia, MCTES (FCT/MCTES). LBM also thanks to FCT/MCTES, for the CEEC-Ind Program Contract.

P3 - Formate dehydrogenase and nitrate: how promiscuous is the enzyme?

Amador, A. (1); Pereira, L. (1); Bertinelli, A. (1); Moura, I. (1); Moura,J. J.G. (1); Maia, L. B. (1)

(1) - LAQV, REQUIMTE, NOVA School of Science and Technology | NOVA FCT, Portugal

Formate dehydrogenases (FDHs) are enzymes that catalyse the reversible two-electron interconversion of formate and CO_2 [1,2]. The active site of the metal-dependent FDHs harbours one molybdenum (or one tungsten) ion coordinated by the cis-dithiolene group of two pyranopterin cofactor molecules, one terminal sulfido group and one sulfur or selenium atom from a cysteine or a selenocysteine residue (depending on the enzyme source). Remarkably, nitrate reductase, an enzyme that catalyses instead an oxygen-atom abstraction reaction (nitrate reduction to nitrite), harbours an active site with the same structure and, in fact, some FDHs (for example the enzyme from Rhodobacter capsulatus [3]) were described to have nitrate reductase activity. In this communication, the ability of the Desulfovibrio desulfuricans FDH (a selenocysteine-containing FDH) to handle nitrate will be revisited.

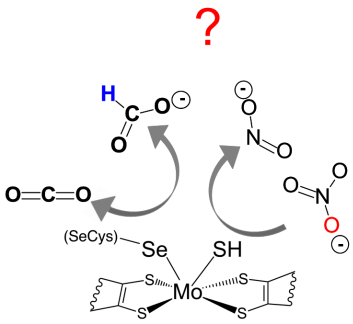


Figure 1: Schematic representation of Desulfovibrio desulfuricans FDH active site.

References

[1] Maia, L., et al. RSC, 2017, 1-80. [2] Maia, L., et al. Carbon Dioxide Utilisation -The Formate Route, 2021, 29-81. [3] Hartmann, T., et al. Biochemistry 2016, 55, 2381-2389.

Acknowledgements

This work was supported by the PTDC/BTA-BTA/0935/2020 project and also by the Associate Laboratory for Green Chemistry - LAQV (UIDB/50006/2020 (DOI: 10.54499/UIDB/50006/2020) and UIDP/50006/2020 (DOI: 10.54499/UIDP/50006/2020)), which are financed by national funds from Fundacão para a Ciência e a Tecnologia, MCTES (FCT/MCTES). LBM also thanks to FCT/MCTES, for the CEEC-Ind Program Contract.

P4 - Exploring Halogen Anisotropy: Impact on Membrane Permeability of Halogenated Drugs

Fortuna, A. (1); Costa, P. J. (2)

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

Halogenation is a common approach to enhance drug-like properties, including membrane permeability. However, halogen atoms can interact with biologically relevant targets, such as proteins and nucleic acids through halogen bonds (XBs) or hydrogen bonds (HBs). This ability arises from the anisotropic electrostatic potential of covalently bonded halogens (X), which creates a positive region (σ-hole) on X opposite to the R–X bond axis. Recent findings [1] suggest that XBs play a role in ligand-membrane interactions, hinting at their potential influence on drug permeation. However, the extent of this effect remains unclear. In this work, six commercially used halogenated drugs - diazepam, bromazepam, clonidine, metolazone, furosemide, and amiodarone - were studied through molecular dynamics simulations combined with umbrella sampling. The permeability coefficients (Pcalc) were derived using the inhomogeneous solubility-diffusion model (ISDM) and a ranking score based on the difference between the maximum and minimum free energies (δGranking). The study revealed very high correlations (R > 0.9) between the calculated and experimental permeability values when using the ISDM model. Incorporating an EP (to emulate σ-hole) facilitated the accurate sampling of XBs without disrupting the sampling of HBs and affecting the Pcalc. These findings highlight the importance of XBs in drug permeation and provide a robust methodology for future studies.

References

[1] Nunes, R. et al. J. Am. Chem. Soc. 2021, 11, 4253–4267.

Acknowledgements

SFRH/BD/146447/20119 (AFortuna), UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 (DOI: 10.54499/UIDP/04046/2020) to BioISI, 2021.00381.CEECIND (DOI: 10.54499/2021.00381.CEECIND/CP1650/CT0004) to PJCosta. EU: project TWIN2PISPA - Twinning for Excellence in Biophysics of Protein Interactions Self-Assembly (GA 101079147).

P5 - Synthesis and structural characterization of new Cu(I)-phosphane complexes for potential therapeutic application in cancer

Fonseca, José M. (1); Figueiredo, Raquel B. (2,3); Robalo, M. Paula (2,3); Morais, Tânia S. (1)

(1) - Centro de Química Estrutural – Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa. (2) – Centro de Química Estrutural – Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa. (3) – Departamento de Engenharia Química, ISEL, Instituto Politécnico de Lisboa.

Copper is an essential metal involved in several fundamental life processes. It is redox active in biological systems due to its readily conversion between its Cu(I) and Cu(II) oxidation states. This redox ability allows copper to act as a key component in many biomolecules with several biological functions [1]. Copper has been recognized as a controlling factor for a variety of processes related to cancer development and progression, particularly in cancer growth and angiogenesis [2].Copper complexes have attracted much interest as potential alternative chemotherapeutics, mainly because they are expected to be less toxic and to overcome platinum resistance by acting through different mechanisms. During last years our group has been involved in the development of new copper(I)-phosphane complexes containing several heteroaromatic ligands which cytotoxicity was found, in most of the cases, higher than that of cisplatin against ovarian, prostate and breast human cancer cell lines [1,3]. Some compounds are shown to be more selective, up to 60-times, towards prostate cancer cells over healthy cells [3]. Herein, we report a new family of Cu(I) complexes of general formula $[Cu(TPTP)_2(LL)][BF_4]$, where TPTP = Tri(p-tolyl)phosphine and LL = N,N; N,S and N,O bidentate ligands (Figure 1). All compounds were fully characterized by different spectroscopy techniques (NMR, FT-IR and, UV-Visible) and their redox behavior in different solvents studied by cyclic voltammetry. The stability of the complexes was evaluated in organic and aqueous media by UV-Visible.

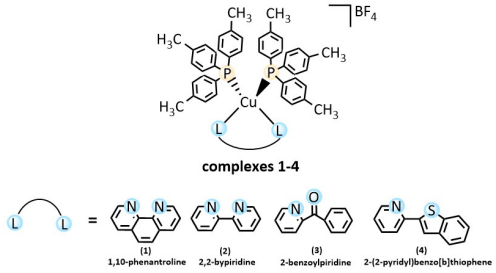


Figure 1: New Copper(I) complexes for potential application in cancer therapy.

References

[1] Morais, T. S., et al., Dalton Trans. 2018, 47, 7819. [2] D'Andrea, L. D., et al., Dalton Trans. 2010, 39, 7625–7636. [3] Machado J. F., et al., Dalton Trans. 2020, 49, 12273.

Acknowledgements

This work was funded by Fundação para a Ciência e Tecnologia (FCT), I.P./MCTES through the projects PTDC/QUI-QIN/0146/2020 (Arrows2cancer, DOI 10.54499/PTDC/QUI-QIN/0146/2020), UIDB/00100/2020 (CQE, DOI 10.54499/UIDB/00100/2020), LA/P/0056/2020 (IMS, DOI 10.54499/LA/P/0056/2020). FCT, POPH and FSE- European Social Funds are acknowledged for the Individual Call to Scientific Employment Stimulus 2022.00028.CEECIND/CP1722/CT0005 (DOI 10.54499/2022.00028.CEECIND/CP1722/CT0005) project.

P6 - Electrochemical Synthesis of Amine-Containing Hypervalent Iodine Reagents

Carvalho, D. F. (1); Lam, K. (2); Marques, M. M. B. (3); Coelho, J. A. S. (1)

(1) - Centro de Química Estrutural - Institute of Molecular Sciences, Faculty of Sciences, University of Lisbon, Portugal. (2) - School of Science, Faculty of Engineering and Science, University of Greenwich, United Kingdom. (3) - LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, Portugal.

Hypervalent iodine reagents (HIR), with iodine in the +III or +V oxidation state, have attracted considerable attention for their utility in metal-free oxidation processes [1]. These compounds have a distinctive feature: a weaker covalent bond between iodine and other atoms compared to conventional covalent bonds. In addition, they possess the remarkable ability to generate electrophilic moieties by reversing the reactivity of established nucleophiles [2]. Among these HIRs, those incorporating transferable nitrogen groups have been investigated. In particular HIR bearing transferable primary amines have been recently disclosed, yet their synthesis presents two synthetic challenges: the need for amine silylation and prior iodine oxidation (Figure 1A) [3]. Here, we present a streamlined approach to the synthesis of amine-containing HIRs. Using a practical, simplified and greener one-pot electrochemical approach based on previously reported procedures for other HIRs (Figure 1B) [4]. The amino HIR compounds are readily prepared by anodic oxidation of cheap and commercially available 2-iodobenzoic acid in the presence of an amine, leading to a faster, scalable and more economically-relevant process [5].

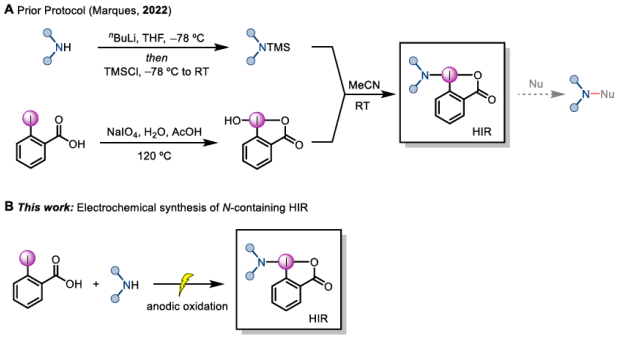


Figure 1: Synthesis of Hypervalent Iodine Reagents with transferable amines.

References

[1] Yoshimura, A., et al. Chem. Rev. 2016, 116, 3328. [2] Hari, D. P., et al. Acc. Chem. Res.2018, 51, 3212. [3] Poeira,D. L., et al. Org. Lett. 2022, 24, 776. [4] Zu, B., et al. Chin. J. Chem. 2021, 39, 627. [5] Leech, M. C., et al. Nat. Rev. Chem. 2022, 6, 275.

Acknowledgements

We thank the Fundação para a Ciência e a Tecnologia (FCT, project 2022.04623.PTDC) for financial support. J.A.S.C. thanks FCT for Scientific Employment Stimulus 2020/02383/CEECIND (https://doi.org/10.54499/2020.02383.CEECIND/CP1595/CT0005).

P7 - Exploring The Role of Sacsin and S100B chaperones in Cytoskeleton Organization in ARSACS Disease

Boasinha, A. S. (1); Murtinheira, F. (1); Macedo, L. (1); Rodrigues, M. (1); Gomes, C. (1); Herrera, F. (1)

(1) - BioISI – Instituto de Biosistemas e Ciências integrativas, Faculdade de Ciências da Universidade de Lisboa, 1749-016, Lisbon, Portugal.

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

References

[1] Murtinheira, F., et al. Cells. 2022, 11, 299. [2] Moreira, G.G., et al. Nat Commun. 2021,12, 6292.

Acknowledgements

We acknowledge the BioISI/FCUL Microscopy Facility, a node of the Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122). FH, ASB and LM were supported by a grant from the ARSACS foundation (Canada). FH, MR and CG were supported by centre grants UIDB/04046/2020 and UID/MULTI/04046/2020 (to BioISI) funded by FEDER funds through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and a TWIN2PIPSA twinning grant from the European Research Council (ID: 101079147). MR and FH were also supported by national funds through Fundação para a Ciência e Tecnologia (Ref. PTDC/FIS-MAC/2741/2021). FM was funded by FCT PhD fellowship ref. SFRH/BD/133220/2017.

P8 - Determination of dextromethorphan and dextrorphan in urine matrices using bar adsorptive microextraction

Fonseca, M. (1); Neng, N. R. (1,2)

(1) - Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749 -016 Lisboa, Portugal. (2) - Laboratório de Ciências Forenses e Psicológicas Egas Moniz, Egas Moniz Center for Interdisciplinary Research, Egas Moniz School of Health Science, Quinta da Granja, 2829-511 Caparica, Almada, Portugal.

Over the past few years, there has been a progressive increase in misuse of medications to induce psychoactive effects similar to illicit drugs, particularly among younger age groups, constituting a serious and growing public health challenge. Dextromethorphan (DXM, figure 1), a cough suppressant agent found in most over-the-counter medications was originally synthesized as a non-narcotic alternative to codeine and other narcotic cough suppressants. At therapeutic doses, DXM is considered a safe drug; however, at supra-therapeutic doses can lead to dissociative states marked by hallucinations, paranoia, and perceptual distortions. Due to its psychoactive properties, DXM has been subject to abuse since its introduction to the market in the 1960s and 1970s.

The present work aims to develop an alternative analytical method for trace determination of DXM and its major metabolite, dextrorphan, in urine matrices, using bar adsorptive microextraction followed by gas chromatography coupled with mass spectrometry analysis. Several parameters affecting the microextraction efficiency were optimized, achieving recoveries above 80% for both compounds.

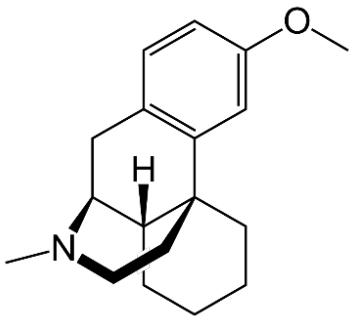


Figure 1: Chemical structure of DXM.

References

[1] Nguyen, L., et al. Pharm. Ther. 2016, 159, 1-22. [2] Conca, A., et al. J. Pharm. Prac. 2012, 25, 13-21.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). CiiEM has provided support through Project 10.54499/UIDB/04585/2020, funded by FCT.

P9 - Novel Oxime Ruthenium-peptide Conjugates For Selective Targeting Of Metastatic Breast Cancer

Sá, M. (1); Morais, T. S. (1)

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

Metastatic breast cancer (MBC) is a highly aggressive subtype that accounts for 15-20% of all breast cancer cases. Unfortunatly, there is no clinical cure for MBC, and available treatments have limited effectiveness and lack of specificity causing severe side effects. Moreover, current treatments are unspecific, often failing to reach metastatic sites [1]. This scenario claims urgency in finding an effective solution. Morais group has been developing novel smart metallodrug delivery systems (SMDS) capable of hunting both primary tumors and metastases, to provide a precision therapy for metastatic cancer that overcomes the limitations of chemotherapeutical in clinical use [2]. These systems comprise a peptide that recognizes with high affinity the fibroblast growth factor receptor (FGFR), often overexpressed by MBC cells, conjugated to a cytotoxic $Ru(II) - (\eta^5 - C_5H_5)$ complex through an linker responsive to the acidic tumor microenvironment, allowing accumulation, site- and time-specific release of the active species into the tumor [2]. One of these systems, containing a hydrazone as a pH-sensitive linker, showed high and selective antiproliferative activity against FGFR(+) BC cells, allied to the controlled release of the cytotoxic ruthenium complex in its active form [2]. In this work, we explored the use of another pH-responsive linker (oximes) to conjugate $Ru(\eta^5 - C_5H_5)$ complexes to the FGFR-targeting peptides, through the bipyridine ligand. Herein we report the synthesis and characterization of two new pH-responsive ruthenium SMDS, as well as the drug release at pH values that mimic the tumor microenvironment and bloodstream.

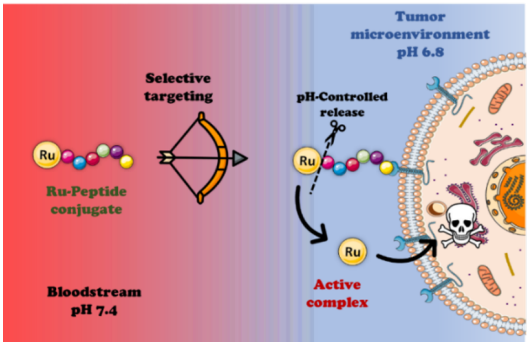


Figure 1: Figure 1. Proposed mechanism of action of the ruthenium-peptide conjugate.

References

[1] Yin, L. et al. Breast Cancer Res. 2020, 22, 1-13. [2] Machado, J. et al. Dalton Trans. 2024, 53, 7645-8054;

Acknowledgements

This work was funded by Fundação para a Ciência e Tecnologia (FCT), I.P./MCTES through the projects PTDC/QUI-QIN/0146/2020 (Arrows2cancer, DOI 10.54499/PTDC/QUI-QIN/0146/2020), UIDB/00100/2020 (CQE, DOI 10.54499/UIDB/00100/2020), LA/P/0056/2020 (IMS, DOI 10.54499/LA/P/0056/2020). M. Sá thanks FCT for his doctoral grant (UI/BD/154814/2023). T.S. Morais thanks FCT, as well as POPH and FSE-European Social Fund for Scientific Employment Stimulus Initiative for the project 2022.00028.CEECIND/CP1722/CT0005 (10.54499/2022.00028.CEECIND/CP1722/CT0005).

P10 - Synthesis of novel N-propargyl glucuronamide potentially bioactive -based nucleosides

Pereira, M. (1); Neto, E. (1); Szilagyi, J. (1); Xavier, N. (1)

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

Synthetic nucleosides, nucleotides, and their analogs/mimetics have garnered significant interest in both organic and medicinal chemistry due to their diverse biological properties, offering promising prospects ranging from antibacterial agents to potential anticancer or antiviral medications [1,2], In our Research Group, we have been dedicated to synthesizing novel glucofuranuronamide-based compounds incorporating various nucleobases and an N-propargyl group, to evaluate their biological profile, specifically their antiproliferative and antibacterial potential. For their synthesis, we employed D-glucofuranurono-6,3-lactone as a precursor, which was converted into an appropriate N-propargyl 1,2-di-O-acetyl glucuronoamidyl donor for subsequent N-glycosylation with a silylated pyrimidine or purine nucleobase. Further synthetic endeavors will concentrate on coupling these molecules to azido pyranoses via triazole formation, as outlined in previous work from our group, to produce novel pseudodisaccharide nucleosides. This approach holds promise for expanding the repertoire of nucleoside analogs with potential therapeutic applications.

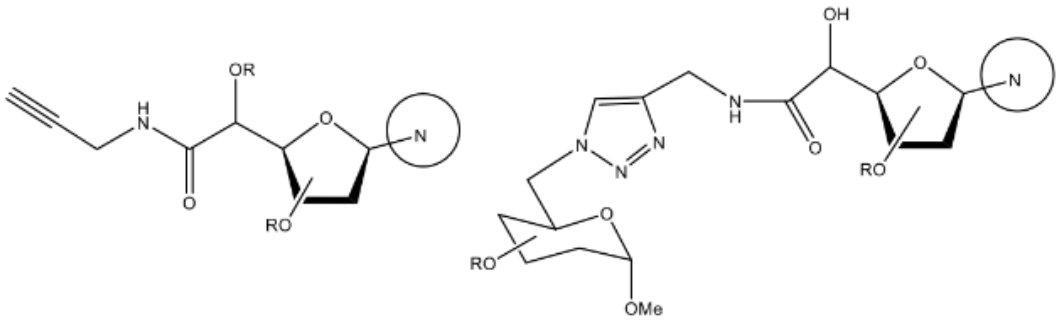


Figure 1: General structures of synthesized nucleosides with and without triazole linkage to a methyl α-D-glucopyranoside moiety

References

[1] Shelton J et al. Chem Rev, 2016, 116:14379. [2] Serpi M et al. J. Med. Chem. 2016, 59:10343. [3] Xavier NM et al. Org Biomol Chem. 2017, 15: 4667-4680. [4] Xavier NM et al. Pure Appl. Chem. 2019, 91:1085. [5] Xavier NM et al. Synlett. 2015, 26: 2663.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). This work was also supported by FCT through project EXPL/MED-QUI/1017/2021 (http://doi.org/10.54499/EXPL/MED-QUI/1017/2021) and grant CEECIND/03881/2018 (https://doi.org/10.54499/CEECIND/03881/2018/CP1553/CT0007).

P11 - SPAX8-related mutations in NKX6-2 form stable aggregates

Ferreira-Peralta, P. (1,2); Murtinheira, F. (1,2); Torres, V. (2); Pinto, F. (2); Rodrigues, M. (2); Herrera, F. (1,2)

(1) - Cell Structure and Dynamics Laboratory, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal. (2) - BioISI – Biosystems Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal.

NKX6-2, a transcriptional factor, influences neurons, oligodendrocytes, and pancreas cell fates, as well as myelin formation and maintenance. Loss-of-function mutations of NKX6-2 are the cause of Spastic Ataxia 8 (SPAX8), a childhood-onset neurodegenerative disease characterized by hypomyelinating leukodystrophy. Our previous work [1] explored SPAX8-related mutations in a NKX6-2-Venus fluorescent protein fusion system, where we noted the aggregation process in most cases. Currently, we are investigating the nature of these aggregates using timelapse microscopy and FRAP assays, observing that SPAX8-related mutations form solid and stable aggregates. In parallel, we are using bioinformatic databases and tools, such as AlphaFold 3.0, to uncover possible insights on NKX homeodomain's interaction with DNA and tinman domain's interaction with the Gro/TLE family. As it was previously observed [2] that the repression complexes form by Gro/TLE family are important for optimal function of multiple proteins of the Sonic the Hedgehog pathway during neuron cell fate, including some members of the NKX family. Our aim is to understand the molecular mechanisms behind SPAX8 and NKX6-2 as whole, as an important step to design a therapeutic strategy for this rare disorder.

References

[1] Ferreira-Peralta, P. et al. BBA-Molecular Basis of Disease. 2024, 1870.1, 166882. [2] Muhr, J., et al. Cell. 2001, 104.6,861-873.

Acknowledgements

We acknowledge the BioISI/FCUL Microscopy Facility, a node of the Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122). FH was supported by a grant from the ARSACS foundation (Canada). FH and MR were supported by centre grants UIDB/04046/2020 and UID/MULTI/04046/2020 (to BioISI) funded by FEDER funds through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and national funds through Fundação para a Ciência e Tecnologia (Ref. PTDC/FIS-MAC/2741/2021). PP and FM were supported by PhD fellowships from FCT (Ref. 2022.14141.BD and SFRH/BD/133220/2017, respectively). This study was supported by the European Union (TWIN2PIPSA, GA101079147).

P12 - Synthesis and characterization of hydrogen-bonded organic frameworks (HOFs) for application in the photocatalytic production of H2

Maçãs, J. (1); Rosa, V. (1); Gomes, C. S. B. (1); Matos, I. (1); Gago, S. (1)

LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University of Lisbon, Portugal.

Nowadays around 95% of global production of hydrogen (H_2) comes from fossil fuels, resulting in H2 with low purity and emission of harmful greenhouse gases. New technological energy approaches without carbon emissions are necessary to rapidly address the challenges of increasing energy needs, global warming, and limited fossil resources. Photocatalytic water splitting for (H_2) production emerges as an environmentally friendly strategy utilizing clean and free sunlight as energy source. However, to be a competitive method, highly efficient, corrosion-resistant, recyclable and low-cost photocatalysts are required [1]. Hydrogen-bonded organic frameworks (HOFs) are a new type of porous crystalline and polymeric materials that is formed through bonds between pure organic ligands, between metal/coordination complexes or between the two types of compounds forming 1D, 2D and 3D structures [2]. In this work, the complex $[Zn(Aryl-BIAN)Cl_2]$ (bis(p-hydroxyphenyl)acenaphthene) and its corresponding HOF were prepared and immobilized in different supports, such as mesoporous silica MCM-41 and commercial NORIT activated carbon GAC 1240W, through functionalization or adsorption. The different materias were characterized by the following techniques Single-Crystal and Powder X-ray Diffraction (SCXRD and PXRD), Fourier Transform Infrared (FTIR), Nuclear Magnetic (NMR) and Diffuse Reflectance Spectroscopies (DRS). Their photocatalytic activity for the production of (H_2) from water splitting was tested using different sacrificial agents [3].

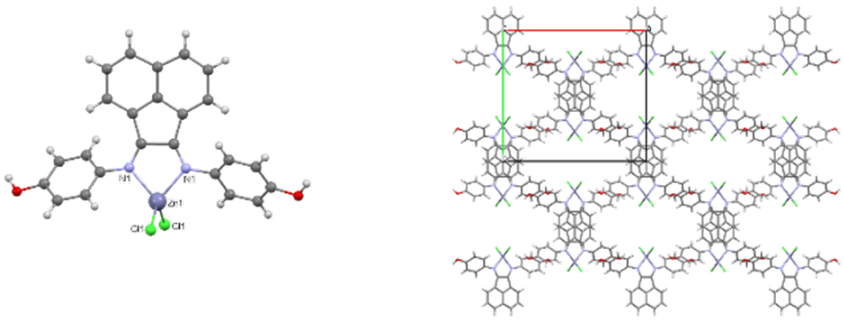


Figure 1: $[Zn(Aryl-BIAN)Cl_2]$ (left) and the corresponding supramolecular arrangement viewed along the c axis (right)

References

[1] Tao, X., et al. Chem. Soc. Rev. 2022, 51, 3561–3608. [2] Yang, J. et al. Chem. Eng. J. 2020, 399, 125873. [3] Koca, A. and Sahin, M. J. Chem. Educ. 2003, 80, 1314–1315.

Acknowledgements

This work was supported by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds.

P13 - Development of new Ruthenium(II)- cyclopentadienyl complexes as potential anticancer agents

Fuchs, S.(1); Marques, B.(1); Morais, T.(1);

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

Cancer is a leading cause of death worldwide, there were an estimated 19.3 million new cases and 10 million cancer deaths in 2020. Most anticancer drugs used in clinical practice are not very effective and frequently have severe side effects, complicating long-term solutions for cancer patients. This supports the critical need for new and innovative treatment options [1]. Ruthenium compounds have attracted considerable attention due to their strong antitumor properties, lower toxicity, and reduced drug resistance compared to platinum-based drugs. Consequently, ruthenium compounds are promising as the next generation of clinically considerable metal-based anticancer drugs [2]. The Ru(II)-cyclopentadienyl complex, [RuCp(PPh_3)(2,2'-bipy)][CF_3SO_3] (TM34), where 2,2'-bipy = 2,2'-bipyridine, showed high cytotoxicity against multiple human cancer cell lines (such as A2780 and A2780cisR ovarian adenocarcinoma, PC3 prostate carcinoma, MCF7 and MDAMB231 breast adenocarcinoma, and HT29 colorectal adenocarcinoma), being more active than cisplatin, especially against resistant cell lines [3,4]. Based on the promising results of TM34 (in the chemical and biological response), we decided to evaluate how a different substituent on the bipyridine ligand affects the physical-chemical properties and activity of the complex. Herein we reported the synthesis of a new compound of general formula [RuCp(PPh_3)L][CF_3SO_3], L = 4,4'-bis(trifluoromethyl)-2,2'-bipyridine. The compound was fully characterized by NMR, UV-Vis, and FTIR spectroscopies. And its stability was also evaluated by UV-Vis spectroscopy, in both organic and aqueous media.

References

[1] Sung, H. et al. CA Cancer J. Clin. 2021, 71, 209-249. [2] Liu, J. et al. ChemComm. 2019, 55, 9904. [3] Santos, F. et al. Biometals 2021, 34,1029-1042. [4] Morais, T. et al. Future Med. Chem. 2016, 8, 527-544.

Acknowledgements

Acknowledgments: This work was funded by Fundação para a Ciência e Tecnologia (FCT), I.P./MCTES through the projects PTDC/QUI-QIN/0146/2020 (Arrows2cancer, DOI 10.54499/PTDC/QUI-QIN/0146/2020), UIDB/00100/2020 (CQE, DOI 10.54499/UIDB/00100/2020), LA/P/0056/2020 (IMS, DOI 10.54499/LA/P/0056/2020). FCT, POPH and FSE- European Social Funds are acknowledged for the Individual Call to Scientific Employment Stimulus 2022.00028.CEECIND/CP1722/CT0005 (DOI 10.54499/2022.00028.CEECIND/CP1722/CT0005) project.

P14 - Oxidation of primary alkyl alcohols to aldehydes catalysed by a copper(I)/TEMPO system

Vaz, M. G. (1); Gomes, C. S. B. (2); Rosa, V. (3)

(1), (2) e (3) - LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology (NOVA FCT), NOVA University Lisbon, 2829-516 Caparica, Portugal.

The selective oxidation of primary alcohol alcohols to aldehydes is an important reaction in the fields of organic and medicinal chemistry with a wide range of applications in pharmaceutical and chemical industries. Unfortunately, the most common methodology for this reaction involves the use of toxic heavy metals, such as chromium, or stoichiometric amounts of reactants. A more sustainable approach for this type of reactions was needed. A solution to this problem was the use of innocuous metals as catalysts combined with molecular oxygen as oxidant. A breakthrough was achieved in 2011 with the development of the Cu(I)/TEMPO system for the aerobic catalytic oxidation of benzylic alcohol to benzaldehyde [1]. Since then, a great deal of research was made to be able to achieve the same results when using alkyl alcohols as substrates. We proposed and applied a family of copper(I) complexes bearing alpha-diimine ligands, bis(imino-aryl)acenaphthene (BIAN), as catalysts to the regioselective oxidation of benzyl alcohols, with whom we achieved great results [2]. Herein we demonstrate the use of this family of complexes and of a new group of iminopyridine Cu(I) based complexes, to oxidize primary alkyl alcohols to aldehydes in mild conditions using air as the oxygen source. Optimization of the catalytic system, including catalyst loading, temperature, and additives composition were undertaken. The complexes were characterized by several spectroscopic methods as well as by single crystal X-ray diffraction, when possible.

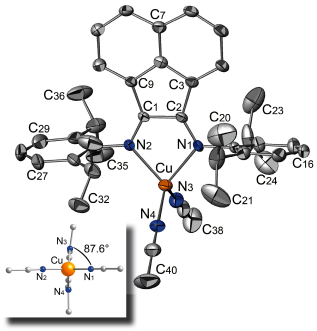


Figure 1: X-ray structure of Cu(I) catalyst.

References

[1] Hoover, J. et al. J. Am. Chem. Soc. 2011, 133, 16901. [2] Rosa, V. et al. Appl. Organomet. Chem. 2023, 37, e7193.

Acknowledgements

This work was supported by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds.

P15 - Nitrous Oxide Reductase: A solution for reducing N2O greenhouse gas emissions

Luciano, C. P. (1,2,3); Moura, I. (3); Pauleta, S. R. (1,2)

(1) - Microbial Stress Lab, UCIBIO, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal. (2) - Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal. (3) - LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.

Nitrous oxide, also known as “laughing gas”, is widely used in healthcare and industry [1]. It is essential to monitor its concentration in these environments, as high direct exposure can affect the function of vitamin B12-dependent enzymes, leading to health problems [2,3]. Furthermore, as a greenhouse gas and the dominant ozone-depleting molecule, N_2O contributes to climate change, increasing the risk of skin cancer and the spread of infectious diseases. Thus, it is essential to develop biotechnological solutions to mitigate N_2O emissions. Nitrous oxide reductase (N2OR) is the only enzyme known to efficiently reduce nitrous oxide to N_2. N2OR is a homodimeric enzyme with two copper centers per monomer: the CuA center, the electron transfer center, and the CuZ center, a rare metal cluster responsible for catalysis. The CuZ center has different forms, CuZ and CuZ*, each with different spectroscopic and catalytic properties [4]. This study aims to characterize N2OR and a variant H583A5 from Stutzerimonas stutzeri. Despite kinetic studies were performed on N2ORs from other organisms, there is still much to learn about Ss N2OR. The wildtype and H583A were isolated, yielding 2 and 0.94 mg of protein/L of growth, and characterized through UV-Vis spectroscopy. In an anaerobic chamber, it was shown for the first time that Ss N2OR is active, with activity increasing with prolonged incubation with reducing agents. Future work will explore the thermal stability of N2OR and their kinetic parameters.

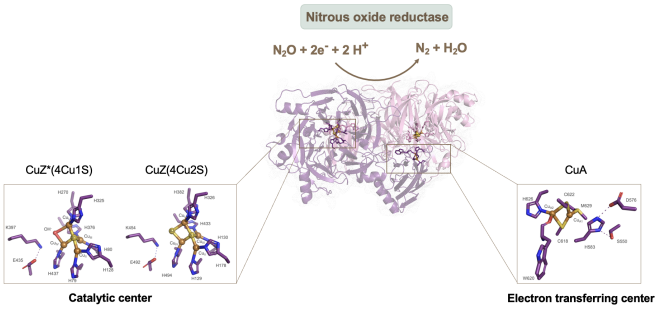


Figure 1: Tridimensional structure of nitrous oxide reductase, highlighting CuZ and CuA center (PDB: 1FWX, 3SBP).

References

[1] Becker, D. E. et al. Nitrous Oxide and the Inhalation Anesthetics. Anesth Prog. 2008, 55(4):124. [2] van Amsterdam, J. et al. R Toxicol Pharmacol. 2015, 73(3):790-796. [3] Pawson, P. and Forsyth, S. SACP. 2008, 83-112. [4] Pauleta, S. R. et al. Coord Chem Rev. 2013, 257(2):332-349.

Acknowledgements

This work was supported by Fundação para a Ciência e Tecnologia by national funds: 2022.01152.PTDC (to IM), CL thank this project for the BI scholarships; UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and UIDB/50006/2020, UIDP/50006/2020 and LA/P/0008/2020 to Associate Laboratory for Green Chemistry-LAQV.

P16 - Unraveling Saliva for Clinical Diagnosis

Vieira, R. (1); Cordas, C. (1); Branco, L. C. (1); Nunes, M. J. (1)

(1) - LAQV-REQUIMTE, Chemistry Department, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal.

Nowadays, biomarkers are widely used for diagnosis, allowing information about human health and well-being. In addition to blood, other fluids have been the subject of interest, such as sweat and saliva, as they are less invasive and more practical methods. For biochemical evaluations, saliva represents an attractive medium, but still relatively little explored, and therefore time should be devoted to its study. The composition of saliva comprises protein, non-protein and microbiological elements, which are of interest for the potential development of a biosensor device that allows the monitoring of human health continuously and in real time. Therefore, the main objective of the project is to identify potential non-protein salivary biomarkers useful for the assessment of human health, more specifically with regard to stress monitoring.The liquid chromatography coupled to TANDEM mass spectrometry analysis was used for the screening and identification of biomarkers in saliva, and a set of 11 small molecules was chosen to evaluate their response. The electrochemical analysis, using the techniques of CV (Cyclic Voltammetry), DPV (Differential Pulse Voltammetry) and SWV (Square Wave Voltammetry), will be used in the future for the detection and quantification of the selected biomarkers, aiming at the future development of sensors that may be useful for the assessment of human health.

References

[1] Nunes, M. J. et al. Sports Medicine - Open. 2021, 7, 8.

Acknowledgements

The authors also thank the support from FCT/MCTES 10.54499/LA/P/0008/2020, 10.54499/UIDP/50006/2020 and 10.54499/UIDB/50006/2020 of the Associate Laboratory for Green Chemistry – LAQV.

P17 - Discovering tears for clinical diagnosis by LC-MS/MS

Costa, M. (1); Nunes, M. J. (1); Noronha, J. P. (1); Branco, L. C. (1)

(1) - LAQV-REQUIMTE, Chemistry Department, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal.

The merging of tear analysis, preventative medicine, clinical diagnosis, and tandem mass spectrometry represents a crucial frontier in the evolution of medical diagnostics and the promotion of personalized healthcare. This exploration is promising, as tears may contain relevant information that can aid in treating medical conditions. Tear collection is a non-invasive procedure, enabling large-scale analysis and offering significant advantages for identifying disease biomarkers. The primary objective of this research is to identify and quantify tear compounds using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to develop a methodology for rapid and versatile screening, tear composition identification, and biomarker selection for clinical diagnosis. LC-MS/MS, utilizing electrospray ionization (ESI) as a source, is an essential technique in this investigation. In this study, eleven biomarkers have been selected for quantification to enhance the understanding of the biochemical composition of tears. This approach represents a significant advance in the potential of tears as a diagnostic tool through the application of advanced mass spectrometry techniques. It contributes to the development of methodologies that allow for the comprehensive analysis of tear composition, a field that has not yet been fully explored, and the identification of their potential biomarkers.

References

[1] Nunes, M. J. et al. Sports Medicine - Open. 2021, 7, 8.

Acknowledgements

The authors also thank the support from FCT/MCTES 10.54499/LA/P/0008/2020, 10.54499/UIDP/50006/2020 and 10.54499/UIDB/50006/2020 of the Associate Laboratory for Green Chemistry – LAQV

P18 - Composite Silica/Polymer and Activated Carbon/Polymer Membranes for Catalytic Applications in Advanced Biorefinery Processes

Machado, B. P. (1); Martins, D. (1); Ventura, M. (1); Bernardo, M. (1); Matos, I. (1)

(1) - LAQV/REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa.

Biomass offers a valuable and eco-friendly alternative to fossil fuels for producing chemicals and products. Developing methods for valorizing and transforming biomass platform molecules, such as levulinic acid and furfural, has great potential for advancing renewable chemical processes within biorefineries, which is why heterogeneous catalysts are receiving increased attention [1]. As we transition towards a circular bioeconomy and aim for commercially viable biorefineries that are both environmentally and economically sustainable, it is essential to maximize the value of all biomass components and products. Advanced technologies like membrane reactors are key to achieving process intensification. We developed two types of catalytic membranes made from composite materials. One set used Polyvinyl alcohol (PVA) as the polymer matrix, while the other used Polymer with Intrinsic Porosity (PIM).These matrices were combined with two different solid porous catalysts, modified MCM-41 and SBA-15, as well as three different activated carbon catalysts derived from two biomass sources (corn cob and ginger lily) and one synthetic source.Mesoporous silica is attractive due to its exceptional thermal and chemical stability, and its surface can be chemically functionalized with acid sites [2]. Porous carbon materials have a high surface area and acidic functional groups on their surfaces.We prepared and optimized composite membranes using various procedures, varying catalyst loading and solvents.This resulted in materials with homogeneous and well-dispersed catalytic phases, that were characterized and tested in furfural acetalization and levulinic acid esterification reactions.

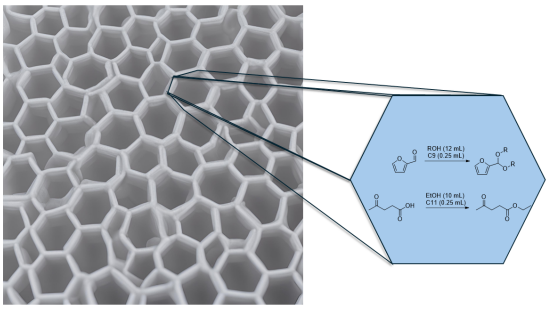


Figure 1: Figure 1. Schematic representation of the active site in an intrinsic mesoporous silica embedded in a membrane, along with the two reactions that are the subject of this study.

References

[1] Dragone, G. et al. Bioresour. Technol. 2020, 302, 122847. [2] Machado,J. et al. 2012, 163, 237-242.

Acknowledgements

This work has been supported by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020, and 2022/05483/PTDC and Inês Matos contract CEECIND/004431/2022

P19 - Exploring the role of JAK3/STAT3 in colorectal cancer

Violante, C. (1); González, B. (2); Alonso, S. (2); Herrera, F. (3)

(1) - Faculdade de Ciências da Universidade de Lisboa, Lisbon, 1749-016, Portugal (2) - Program of Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), 08916 Badalona, Spain (3) - BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências da Universidade de Lisboa, Lisbon, 1749-016, Portugal

Colorectal cancer (CRC) has low immunogenicity, hindering the response of these tumours to immunotherapy. This characteristic feature is attributed to mutational burden and factors in the tumour microenvironment (TME). The signal transducer and activator of transcription 3 (STAT3) plays a crucial role in shaping the TME, being persistently active in 70% of solid tumours, and promoting survival, proliferation, and immunosuppression within the tumours. Epigenetic alterations are a hallmark of cancer, notably in CRC, and could be relevant for the transcriptional activity of STAT3. This study investigates the impact of epigenetic DNA methylation in genes of the STAT3 pathway on CRC's immunogenicity. The research involved a DNA methylation analysis in diverse CRC cell lines and a small intestine cell line linked to the STAT3 pathway, focusing on JAK3, which was identified through computational analysis to have promoter methylation. Specifically, HCT116 and SW480 were found to be more methylated compared to LS174T and HT29. Furthermore, CRC cell lines with varying JAK3 expression levels were characterized, and the effects of demethylation using a chemical agent were assessed. In contrast to the expected significant increase in expression due to demethylation in cell lines with higher methylation status, the results did not confirm this hypothesis. This study will also examine protein expression in JAK3, activated (Y705-phosphorylated) STAT3, and total STAT3. The findings may shed light on the potential of targeting the JAK3-STAT3 signalling pathway to enhance immunotherapy efficacy in CRC.

References

[1] Lin, Q. et al. AJP. 2005, 167, 969–980. [2] Sillo, T. et al. BJS. 2019,106,1283–1297

Acknowledgements

This work has been supported by grants to S. Alonso from the Instituto de Salud Carlos III (FIS PI21/01766), Spanish Ministry of Science, Innovation and Universities, FEDER, and by Fundación Mutua Madrileña (AP174232020). C. Violante was the recipient of an Erasmus+ fellowship for Higher Education Learning Agreement for Traineeships

P20 - Unveiling Pyruvate:quinone oxidoreductase (PQO) of Staphylococcus aureus

Santos, D. (1); Ressaissi, A. (1); Calisto, F. (1); Pereira, M. (1)

(1) - University of Lisbon, Faculty of Sciences, Department of Chemistry and Biochemistry, and BioISI – Biosystems Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

According to the Global Burden of Disease, from all microorganisms with resistance to antibiotics, Staphylococus aureus is the responsible for the most deaths of individuals older than 15 years old, and has the highest geographical distribution, being the leading bacterial cause of death in 135 countries. Therefore, new methods are necessary to combat this pathogen. As the energy metabolism is essential to all life, the study of proteins involved in the energy metabolism and its mechanisms can lead to new approaches to reduce S. aureus severity. The aim of this project is to study the pyruvate:quinone oxidoreductase (PQO), a protein hypothesized to be involved in the respiratory chain of S. aureus, through characterization involving biochemical, biophysical and and cellular methodologies. We cloned and expressed the gene encoding PQO in E. coli cells and purify the protein with a purity yield above 90%. To our knowledge we were the first group to succeed in performing kinetics assays and determining the kinetics parameters with pure protein, using different electron acceptors. Most importantly, we confirmed that PQO has as substrate an analogue of menaquinone, the physiological quinone present in the respiratory chain of this pathogen. Additional we also produced a knockout mutant, ΔPQO, which enabled us to analyze the impact of PQO and its role in the cell. We expect that our results will lead to a better understanding of PQO and its mechanisms to possibly unveil new inhibitors that may decrease S.aureus pathogenicity.

References

Acknowledgements

The work was funded by Fundação para a Ciência e a Tecnologia (PTDC/BIA-BQM/2599/2021, DOI: 10.54499/PTDC/BIA-BQM/2599/2021 to MMP). The project was further Work supported by UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 (DOI: 10.54499/UIDP/04046/2020) Centre grants from FCT, Portugal (to BioISI).

P21 - Exploring tau and amyloid-β cross-interactions in Alzheimer's Disease

de Freitas, D. P. (1,2); Gomes, C. M. (1,2)

(1) - BioISI — Biosystems Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal. (2) - Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

A hallmark of Alzheimer's Disease (AD) is the emergence of proteinaceous aggregates in the brain, mostly composed by amyloid β (Aβ) in extracellular plaques, and tau in intracellular tangles, with protein dysregulation starting from earlier stages. Despite their different localization, tau exits cells and spreads throughout the brain, highlighting the extracellular milieu as a space where potential interactions between tau and Aβ might occur, that could influence the progression of the pathology. Furthermore, S100B, a late-stage alarmin, can act as an early-stage extracellular chaperone against tau [1] and Aβ2 [2] aggregation. Thus, we explored plausible cross-interactions between tau and Aβ in vitro, and the effect of S100B in this context. As a tau model we used the tau fibril core (TADC, $tau_{306-378}$), which aggregates without heparin, making it closer to in vivo settings. It was evidenced that TADC aggregation was accelerated in the presence of Aβ42 in a concentration-dependent manner but Aβ42 aggregation was inhibited by tau. S100B showed a dual-behaviour on TADC aggregation, accelerating it at lower ratios and fully inhibiting it at equimolar ones, though this inhibition was not complete when heparin was present, suggesting that its presence conditions the type of species generated, which were also differentially detected by the amyloid-sensitive fluorophores ThT and X-34. Lastly, in mixed conditions with Aβ42, the inhibitory effect of S100B was lost, hinting to a competition for the S100B dimer. Overall, these results underline the complex interplay of events taking place in early AD scenarios, stressing the need to explore them further to better understand AD progression.

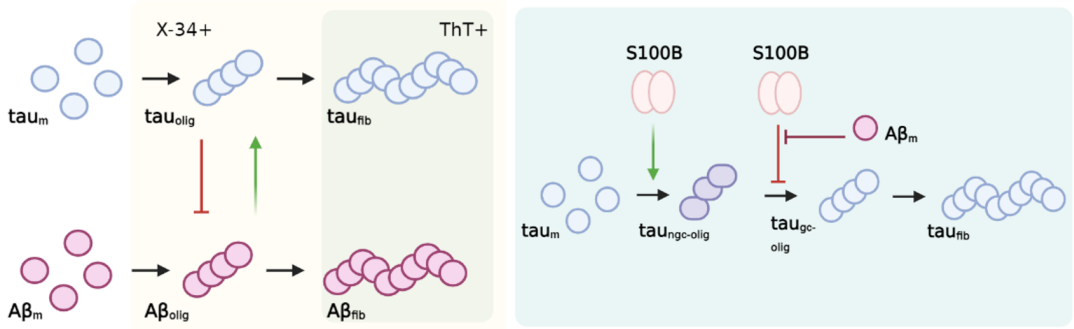


Figure 1: Aβ aggregation was inhibited by tau while tau aggregation was enhanced by Aβ. S100B has a dual-effect over TADC aggregation, with Aβ interfering if present.

References

[1] Moreira, G. et al. Nat Comm. 2021, 12(1):6292. [2] Cristóvão, J. et al. Sci Adv. 2019, 4(6):eaaq1702.

Acknowledgements

EU for funding Twinning Grant EU-TWIN2PIPSA/GA101079147, FCT/MCTES (Portugal) for funding UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/MULTI/04046/2020 (DOI: 10.54499/UIDP/04046/2020) (BioISI) and PhD grant to DPF (DOI: 10.54499/2023.03769.BD) and Agilebio for funding LabCollector Scientific Award 2021 (CMG).

P22 - Acylations as modulators of mitochondrial beta oxidation proteins

Domingos, R. (1); Ribeiro, J. V. (1), Henriques, B. J. (1)

(1) - BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Metabolic regulation involves a complex interplay of genomic, proteomic and metabolic adjustments within cells. A particular group of non-enzymatic post-translational modification (PTMs) known as acylations, such as succinylation and glutarylation, have emerged as important regulators of mitochondrial enzymes [1]. The extent of acylations is closely associated with the accumulation of intermediate metabolites such as Succinyl-CoA and Glutaryl-CoA, that occur under certain conditions such as caloric restriction and in several metabolic disorders, creating a unique scenario for anomalous protein acylation [2]. Although several studies have identified enzymes that are acylated, and sirtuin substrates (enzymes responsible for modification reversion), the impact at the protein structural and functional level of these modifications remains to be fully addressed. Taking advantage of our know-how with mitochondrial beta oxidation enzymes, we have been studying acylation's impact in different proteins using biochemical and biophysical techniques. We have shown that glutaryl-CoA dehydrogenase (GCDH) is prone to high levels of glutarylation, due to increased glutaryl-CoA production stimulated by lysine catabolism, which diminishes enzyme activity and is regulated by sirtuin5 (Sirt5) [3]. In contrast Medium Chain Acyl-CoA Dehydrogenase (MCAD) and Electron Transfer Flavoprotein (ETF) are prone to succinylation. Interestingly, succinylation increases MCAD activity but decreases ETF activity, although no major differences were shown in neither proteins' structure nor stability. Further, Sirt5 incubation reverts succinylation and brings function of both proteins to unmodified levels.

References

[1] Wagner, G. R. et al. Cell Metab. 2017, 25(4): p. 823-837. [2] Pougovkina, O. et al. J Inherit Metab Dis. 2014, 37(5): p. 709-14. [3] Bhatt, D. P. et al. J Biol Chem. 2022, 298(4) 101723.

Acknowledgements

Fundação para a Ciência e Tecnologia (FCT/MCTES, Portugal) for grants: DOI: 10.54499/SFRH/BD/147202/2019 (to J.V.R.), DOI:10.54499/2021.02218.CEECIND/CP1650/CT0008 (to B.J.H), DOI 10.54499/PTDC/BIA-BQM/29963/2017 and PTDC/BBB-BQB/5366/2014 (to B.J.H), centre grants DOI: 10.54499/UIDB/04046/2020 and DOI: 10.54499/UIDP/04046/2020 (to BioISI). This research is partially funded by the European Union (TWIN2PIPSA, GA 101079147). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

P23 - Novel phosph(on)ate-containing isonucleotide analogs based on D-glucopyranuronamide units endowed with antibacterial effects in S. pneumoniae

Manuel, D. M.(1); Moreira, T.(1); Filipe, S.(2); Xavier, N.M.(1)

(1) – Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa. (2) – UCIBIO- Applied Molecular Biosciences Unit - Associate Laboratory i4HB, NOVA FCT.

The development of molecules containing D-glucuronamide moieties is a promising approach in the search for new carbohydrate-based bioactive compounds, given the variety of biological activities reported for both natural or synthetic derivatives containing this glycosyl unit, including antimicrobial or anticancer effects [1].

The synthesis of glucuronamide derivatives also enables structural variations in a gluco-configured template, which is an important aspect for bioactivity tuning, namely at C-6, through N-substitution with various kinds of moieties, and at C-1, using typical methodologies for anomeric functionalization.

In the context of our ongoing interest in the search for new synthetic bioactive D-glucuronamide-containing molecules and encouraged by our previous findings showing the biological profile of N-dodecyl glucuronamide-based nucleosides [2-5], in this communication we disclose the synthesis of novel isonucleos(t)ide derivatives, containing (purinyl)alkyltriazole or alkylpurine moieties at C-6 and an anomerically-N-linked 1,2,3-triazole phosphonate and phosphate system. D-Glucofuranuronolactone was used as a precursor in the synthetic pathways which included key steps such as amidation, purine alkylation, furanose to pyranose isomerization, azidation, azide-alkyne 1,3-dipolar cycloaddition and Staudinger reaction. Antibacterial evaluation revealed the significant activities of some isonucleotides against a Streptococcus pneumoniae clinical strain, turning them promising “hits” for further studies.

References

[1] Xavier, N. M. and Fortuna, A. In: Reedijk J (Ed.), Chem. Eng. Sci. 2019, Waltham, MA: Elsevier. [2] Xavier, N. M. et al. Org Biomol Chem. 2017,15:4667. [3] Pereira, R. G. et al. EurJOrgChem. 2018, 2667: 2681 [4] Xavier, N. M. et al. PureApplChem. 2019, 91:10845. [5] Moreira, T. et al. ChemMedChem. 2024, 9:e202300608

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). This work was also supported by FCT through project EXPL/MED-QUI/1017/2021 (http://doi.org/10.54499/EXPL/MED-QUI/1017/2021) and grant CEECIND/03881/2018 (https://doi.org/10.54499/CEECIND/03881/2018/CP1553/CT0007). D. M. Manuel also thank INAGBE-Angola and Instituto Superior de Ciências de Educação da Huíla

P24 - Exploitation of the synthesis of potential sugar diphosphate and nucleotide mimetics containing sulfonylacetate systems

Duarte, P. (1); Moreira, T. (1); Xavier, N. M. (1)

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

Nucleoside and nucleotide analogs represent an important group of molecules in bioorganic and medicinal chemistry. Known for their ability to inhibit nucleotide-dependent processes, such as nucleic acid synthesis, cell division, signaling and metabolism, these groups of molecules have wide application for treating cancer and viral infection [1]. Their potential as antibacterials is also well documented [2,3]. Recent FDA approvals for drugs like remdesivir and molnupiravir highlight their significance in combating COVID-19 [4]. However, with the emergence of chemotherapeutic resistance and the low bioavailability of these molecules [1], it is imperative to design and synthesize novel bioactive nucleoside/nucleotide-like structures capable of overcoming these limitations, offering improved and alternative mechanisms of action.

In this context, we will present our synthetic work towards sulfoacetate-linked pseudodisaccharide nucleosides designed as mimetics of nucleoside diphosphate sugars. The synthesis of these compounds follows a convergent approach, in which partially protected nucleosides and sugar (chlorosulfonyl)acetates are separately synthesized and are subsequently coupled together. The synthetic strategy involved several steps, including monosaccharide protection-deprotection steps leading to differently substituted monosaccharide derivatives, azidation, N-glycosylation, chlorosulfonylacetylation, and reduction.

References

[1] Jordheim L. P. et al. (2023) Nat. Rev. Drug. Discov. 2023, 12:447-464. [2] Serpi, M. J Med Chem. 2016, 59: 10343. [3] Thompson, J. M. et al. Front Microbiol. 2019, 10: 952. [4] Aboul-Fotouh, S. et al. Virol J. 2023, 20:241

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). This work was also supported by FCT through project EXPL/MED-QUI/1017/2021 (http://doi.org/10.54499/EXPL/MED-QUI/1017/2021) and grant CEECIND/03881/2018 (https://doi.org/10.54499/CEECIND/03881/2018/CP1553/CT0007).

P25 - Novel phosphoramidate-containing isonucleotide analogs synthesized using D-glucopyranuronamide units

Parente, L. (1); Manuel, D. M. (1); Abid, A. (1); Nelo, M. (1); Xavier, N. M. (1)

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

Nucleoside and nucleotide analogs are important groups of molecules in (bio)organic and medicinal chemistry, owing to their ability to mimic their physiological counterparts and exhibit biological activities of therapeutic application, among which anticancer and antiviral effects. In our Research Group, we have been dedicated to the development and synthesis of a variety of novel types of bioactive nucleos(t)ide analogs, by exploring the installation of uncommon glycosyl units, phosphate mimetic groups and substituted purine derivatives or other nitrogeneous heteroaromatic systems. D-Glucuronamide-based nucleosides are among the molecules developed in our Group, and various groups of compounds showed relevant bioactivities, including in-vitro anticancer effects similar or close to those exhibited by standard drugs. Motivated by our previous findings, we explored the synthesis of novel types of isonucleotidic frameworks based on a D-glucuronamide unit for further evaluation of their therapeutic interest. The groups of molecules whose synthesis will be presented herein, comprise a (purinyl)alkyltriazole and alkylpurine moieties at C-6 of the glucuronamide unit and an anomerically-N-linked phosphoramidate group. The synthetic pathways used D-glucofuranuronolactone as a precursor and key steps such as amidation, purine alkylation, azide-alkyne 1,3-dipolar cycloaddition, azidation, furanose to pyranose isomerization, and Staudinger reaction.

Figure 1: Figure 1. General structure of the synthesized molecules

References

[1] Jordheim, L. P. et al. Nat Rev Drug Discov. 2013, 12:447. [2] Xavier, N. M. et al. Org Biomol Chem. 2017, 15:4667. [3] Xavier, N. M. et al. Pure Appl Chem. 2019, 91:10845. [4] Moreira, T. et al. ChemMedChem. 2024, 19:e202300608.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). This work was also supported by FCT through project EXPL/MED-QUI/1017/2021 (http://doi.org/10.54499/EXPL/MED-QUI/1017/2021) and grant CEECIND/03881/2018 (https://doi.org/10.54499/CEECIND/03881/2018/CP1553/CT0007). D. M. Manuel also thank INAGBE-Angola and Instituto Superior de Ciências de Educação da Huíla.

P26 - A precise method to estimate the enthalpies of combustion of biodiesels

Rodrigues, R. I. (1); Bernardes, C. E. S. (1); Minas da Piedade, M. E. (1); Borges dos Santos, R. M. (1,2)

1Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal 2Centre of Marine Sciences (CCMAR/CIMAR LA), Departamento de Química e Farmácia, Faculdade de Ciências e Tecnologia, Universidade do Algarve, 8005-139 Faro, Portugal

Air pollution has, in the last decades, emerged as a significant global challenge, largely due to the excessive use of fossil fuels in the transportation sector. As a result, there is currently an urgent need of transition to more sustainable energy sources. Biodiesel is a mixture of fatty acid methyl esters (FAME) obtained by transesterification of oils from renewable sources with methanol. This is a promising alternative to conventional fuels, given the low emission of greenhouse gases [1] and the simplicity inherent to the production and use of biodiesel. The combustion enthalpy, $\Delta \mathrm{c}H\mathrm{m}$, is the most important physical property of any fuel, as it allows the evaluation of its energetic efficiency. As its experimental determination can be demanding, there is a compelling need to develop reliable and efficient methods for the estimation of FAME's $\Delta \mathrm{c}H\mathrm{m}$. Several methods have been proposed, but they have all been found to be limited or imprecise. Benson's method [3] is one of the most rigorous group additivity methods available. It is particularly suitable to address FAMEs, given the structural simplicity of the molecules, which allows precise estimations with a very limited number of groups. In this work, an algorithm was developed for the estimation of FAMEs $\Delta \mathrm{c}H\mathrm{m}$ based on Benson's method, and the $\Delta \mathrm{c}H\mathrm{m}$ of key FAMEs was estimated. To validate the algorithm, the enthalpy of formation, $\Delta \mathrm{f}H\mathrm{m}$, of the same molecules was determined. The obtained results agreed with those given by the algorithm within the so-called chemical accuracy of 4 kJ mol^{-1}.

References

[1] Bonomi, A. et al. Phase 2, Part 1: FAME and HVO/HEFA. [2] Cohen, N. and Benson, S. W. Chem. Rev. 1993, 93 (7), 2419–2438.

Acknowledgements

This work was funded by FCT – Fundação para a Ciência e a Tecnologia, Portugal, through projects UIDB/00100/2020 (DOI 10.54499/UIDP/00100/2020), UIDP/00100/2020 (DOI 10.54499/UIDP/00100/ 2020), LA/P/0056/2020 (DOI 10.54499/LA/P/0056/2020), UIDB/04326/2020 (DOI:10.54499/UIDB/04326/2020), UIDP/043

P27 - Missing Information On The Kinetic Profile Of T-BuCl: Is There Anything We Can Do?

Pinção, A. (1,2); Moreira, L. (1,3); Elvas-Leitão, R. (1,4); Martins, F. (1,2)

(1) - Centro de Química Estrutural - Institute of Molecular Sciences, Universidade de Lisboa. (2) - Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa. (3) - Instituto Superior de Educação e Ciências, ISEC Lisboa. (4) - Departamento de Engenharia Química, Instituto Superior de Engenharia de Lisboa, IPL.

The study of 2-chloro-2-methylpropane (t-BuCl) solvolysis has a rich history, dating back to almost 90 years ago [1], but to this day, remains as a reference reaction in kinetics [2]. However, despite this long history, the kinetic profile of t BuCl vs. xw (water mole fraction) remains somewhat elusive. Information is either scattered throughout the literature or incomplete, particularly for aqueous mixtures with various co-solvents. For many common solvents, such as DMSO, data is notably absent. To address this gap, several strategies were used to obtain a comprehensive kinetic profile of this substrate for the set of 13 binary aqueous mixtures under study: i. Collate and confirm existing results from literature for the target mixtures; ii. Experimentally determine t-BuCl rate constants (k) when kinetic data were missing; iii. In cases where reactions were predicted as being very slow, compute k values for t BuCl either by interpolation from t-BuBr kinetic profile or by temperature extrapolation (to 25 ºC) of t- BuCl kinetic information available for the same mixtures at other temperatures. These systematic strategies have collectively contributed to a far-reaching understanding of t-BuCl solvolysis, sealing gaps in the literature and enhancing our knowledge of this reference reaction.

References

[1] Hughes, E. D. et al. J Chem Soc (Resumed). 1935, 244-255. [2] Moreira, L. et al. J Mol Liq. 2019, 291, 111244.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

P28 - Synthesis of butane-2,3-diacetal (BDA)-protected derivatives of functionalized monosaccharides

Gonçalves-Pereira, R. (1); Nelo, M (1); Xavier, N. M. (1)

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

Selective hydroxyl group protection in polyhydroxylated molecules is a continuing challenge in organic chemistry. In particular, in carbohydrate chemistry, the selective protection of vicinal diols is often required. For 1,2-cis diols, several acetal protecting groups have been developed, while for 1,2-trans diequatorial diols, fewer protection methods were reported, and those include the use of 1,3-disiloxanyl [1] and 1,2-diacetal protecting groups [2]. Among the latter, the butane 2,3-diacetal (BDA) system has been shown to be stable towards a broad range of reagents and can be cleaved under acidic conditions.

In the context of our ongoing interest in the development of novel bioactive carbohydrate-based structures, especially nucleos(t)ide analogs, we present herein the synthesis of BDA-protected derivatives of functionalized monosaccharides, namely N-substituted glucuronamides and fluorinated monosaccharide derivatives, which are types of moieties recognized for their biological potential [3,4]. The introduction of the BDA-fused system can also reduce conformational flexibility of the molecules, which is an important aspect to consider in the development of more selective bioactive molecules.

The synthetic work included key reactions such as opening of glucofuranuronolactone with an amine, furanose to pyranose isomerization, selective monosaccharide fluorination and acid-mediated BDA protection.

References

[1] Johnsson, R. Carbohydr. Res. 2012, 353:92. [2] Ley, S.V. et al. Chem. Rev. 2001,101:53. [3] Linclau, B. et al. Chem. Soc. Rev. 2020 ,49: 3863. [4] Xavier, N. M., Fortuna, A. In: Reedijk J (Ed.), Module in Chemistry, Molecular Sciences and Chemical Engineering. 2019, Waltham, MA: Elsevier.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020). This work was also supported by FCT through project EXPL/MED-QUI/1017/2021 (http://doi.org/10.54499/EXPL/MED-QUI/1017/2021) and grant CEECIND/03881/2018 (https://doi.org/10.54499/CEECIND/03881/2018/CP1553/CT0007).

P29 - Synthetic Approaches and Computational Studies of Naturally Occurring Phenolic Carbohydrate Esters as Lead Pharmaceutics

Maciel, V. (1); Petrova, K. (1); Georgieva, M. (1)

(1) LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

Carbohydrates are indispensable components for living systems, providing nourishment, energy and even contributing to the treatment of various diseases. However, there are still few drugs containing carbohydrates on the market and more research efforts need to be done, particularly on compounds based on sucrose [1,2]. Phenolic Sucrose Esters (PSEs) are a class of bioactive compounds, traditionally employed in folk medicine and sourced from various plants, known for their anti-proliferative, antioxidant, anti-inflammatory, and α-glucosidase inhibitory properties. Very few of these have been obtained synthetically, which, combined with the milligram quantities isolated from plants in pure form, has prevented their use in pharmacology [3,4]. Initially identified in Raphanus sativus, PSEs have since been discovered in various plant species frequently used in alternative medicine, such as Veronicastrum sibiricum, Musa acuminata, among others. [5] With this project, we explored one-step selective chemical methodologies (Mitsunobu conditions) to synthesize 24 biologically active sucrose esters with very promising applications (Scheme 1). A part of the project includes a computational estimation of the radical scavenging effects through different reaction paths - hydrogen atom transfer (HAT), single electron transfer (SET) and radical adduct formation (RAF). Then, we will perform in-vitro studies to experimentally determine the antioxidant activities in order to compare the results and find structure-activity relationships (SAR). We have successfully synthesised the target compounds with yields up to 33%, succeeding the first synthesis of 6-O-benzoyl sucrose ester and others.

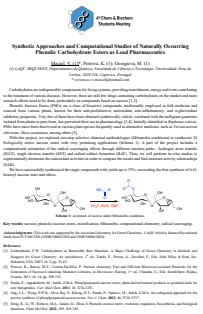


Figure 1: Veronica$_{M}aciel_{p}oster.pdf$

References

Full references on "Abstract image" (I couldn't fit them here).

Acknowledgements

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).

P30 - Hollow Fiber Microextraction (HFμE) for the Determination of Trace Levels of Pyrethroids in Water Samples

Miguéis, R. (1); Nogueira, J. M. F. (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;

Pyrethroids are chemical compounds belonging to the class of insecticides, currently used indiscriminately in the agricultural sector, as they have low toxicity characteristics compared to previously used pesticides. However, widespread use has promoted progressive accumulation in soils and surrounding waters, increasing their toxic potential and affecting the surrounding ecosystems. In this way, exposure to this environmental contamination may be harmful to public health, so according to national and European legislation it is essential to establish expeditious and sensitive analytical methodologies to reduce the consumption of food and water contaminated by pesticides that exceed the permitted limits [1,2].

The present work aims to apply a new analytical approach based on the principles of green chemistry. Hollow fiber microextraction, in combination with gas chromatography coupled to mass spectroscopy (HFμE/GC-MS), will be proposed to monitor trace levels of seven pyrethroids (allemrin, tetramethrin, bifenthrin, phenpropatrin, lambda-cyhalothrin, cypermethrin, and fenvalerate) in water matrices.

The HFμE methodology was developed and optimized by evaluating several relevant parameters during the microextraction (type of extractor solvent, equilibrium time, agitation speed, organic modifier, and ionic strength) and back-extraction stages. The recoveries obtained diverged from 65.2 % to 105.1 % with RSD < 10.2 %, varying depending on the pyrethroid involved. In addition, it is also our intention to apply this optimized methodology and, subsequently, apply it to real matrices (surface, ground and drinking water).

References

[1] Ahamad, A.,Kumar, J. Pyrethroid pesticides: An overview on classification, toxicological assessment and monitoring. JHM Advances, 2023, 100284; [2] Gajendiran, A., Abraham, J. An overview of pyrethroid insecticides. Front. Biol., 2018, 13(2), 79–90;

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by Fundação para a Ciência e a Tecnologia (FCT) through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

P31 - Interaction of Pyk2 and BIN1 proteins that are both associated to genetic risk factors of Alzheimer's disease

Lucy Martin (1,2), Gang Peng (1,2), François-Xavier Cantrelle (1,2), Emmanuelle Bolle (1,2), Alexis Verger (1,2), Clément Danis (1,2,3), Elian Dupré (1,2), Pierre Dourlen (2), Isabelle Landrieu (1,2)

(1) CNRS EMR 9002 Integrative Structural Biology F-59000 Lille, France (2) Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Risk Factors and Molecular Determinants of Aging-Related Diseases, F-59000 Lille, France (3) Univ. Lille, Inserm, CHU Lille, U1172 - LilNCog - Lille Neuroscience Cognition, F-59000 Lille, France

While late-onset Alzheimer's disease (AD) is predominantly considered sporadic, a significant genetic predisposition exists, with over 70 loci, including BIN1 and Pyk2, associated with AD [1]. However, the roles of these risk factors remain unclear. This study investigates the interaction between Pyk2 and the BIN1-SH3 domain, which facilitates protein-protein interactions through proline-rich motifs. Pyk2 has such motifs within a flexible proline-rich linker. To elucidate Pyk2's role in AD, its interaction with BIN1-SH3 was compared to Fak1, a paralog not linked to AD. NMR signal assignment confirmed the disordered nature of the Pyk2 and Fak1 linkers, with a helical region observed by Alphafold and NMR [2]. NMR mapping identified interaction sites between BIN1-SH3 and two proline-rich peptides in Pyk2-LK2, but only one in Fak1-LK2, despite similar sequences. Interactions with BIN1 isoforms 1 and 9 were confirmed via 2D NMR spectra, emphasizing BIN1's SH3 domain involvement. NMR, ITC, and SPR were used to determine interaction affinities. Kd values from NMR and ITC were consistent with typical SH3 domain interactions, while SPR discrepancies were attributed to different interaction contexts; SPR analyzed entire Pyk2/Fak1 LK2 with SH3, whereas NMR and ITC focused on specific peptides. Pulldown experiments with GST-fused Pyk2 and Fak1 domains confirmed their interactions with BIN1's LK2, supporting the findings. This study links two genetic risk factors, BIN1 and Pyk2, through direct interaction within the same pathway, possibly related to the focal adhesion complex. This connection provides new hypotheses about their roles in AD pathophysiology.

References

[1] Bellenguez, C et al. New insights into the genetic etiology of Alzheimer's disease and related dementias, Nat Genet. 2022, 54, pages 412–436 [2] Momin, A et al. PYK2 senses calcium through a disordered dimerization and calmodulin-binding element, Commun Biol. 2022, 5, Article number: 800

Acknowledgements

This study was supported by DISTALZ (Development of Innovative Strategies for a Transdisciplinary approach to Alzheimer's disease ANR-11-LABX)

P32 - Quantification of Six Phosphatidylethanol Homologues in Whole Blood with Minimized Phospholipid Interference

Maria, Marisa (1); Neng, Nuno (1,2); Berg, Thomas (3)

(1) - Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749 -016 Lisboa, Portugal (2) - Laboratório de Ciências Forenses e Psicológicas Egas Moniz, Centro de Investigação Interdisciplinar Egas Moniz, Egas Moniz School of Health and Science, Campus Universitário, Quinta da Granja, 2829-511 Caparica, Almada, Portugal (3) - Department of Forensic Sciences, Division of Laboratory Medicine, Section of Drug Abuse Research, Oslo University Hospital, P.O. Box 4950 Nydalen, N-0424, Lovisenberggt. 6 Oslo 0456, Norway

Alcohol consumption is linked to various health risks, injuries, and even death, and has significant social and economic consequences worldwide. Phosphatidylethanol (PEth), stands out as a promising group of direct alcohol biomarkers, with a notably longer half-life in blood compared to ethanol. These biomarkers can be measured to predict various patterns of alcohol consumption [1]. The objective of this study was to develop and validate an accurate and precise LC-MS/MS method for the determination of six PEth homologues in whole blood, while minimizing interference from unwanted phospholipids. Different organic solvent mixtures for liquid-liquid extraction were investigated aiming to achieve optimal recovery of PEth homologues while eliminating lyso-phospholipids and other interfering phospholipids. For the instrumental analyses and chromatographic separation, an LC-MS/MS with BEH C18 column was used. The mobile phase consisted of 0.025% ammonia in Type I water, pH 10.7, as solvent A, and methanol as solvent B [2]. After method optimization, it was found that the mixture of heptane/2-propanol (80:20, v:v) provided the lowest phospholipid background, satisfactory recovery of all six PEth homologues, and the best signal-to-noise values. The method validation was performed by using blank whole blood as matrix in calibrators and QC samples. Lower limit of quantification was 10 nM for all compounds. The extraction recoveries obtained were within 37-51% and no matrix effects were observed. Quantification of 22 authentic blood samples showed that the developed LC-MS/MS method is sensitive, precise and accurate for the determination of the six PEth homologues in whole blood [3].

References

[1] Jones AW (2010) Forensic Sci Int. 200(1–3):1–20. [2] Maria MH, Jørgenrud BM, Berg T (2022) J Chromatogr A 1684:463566. [3] Maria M, Neng NR, Berg T. (2023) J Chromatogr A. 1711:464451

Acknowledgements

This work was financed by national funds through Fundação para a Ciência e a Tecnologia (FCT), under the Project UIDB/04585/2020. Centro de Química Estrutural is a Research Unit funded by FCT through projects (PIDDAC - UIDB/00100/2020 and UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020. Marisa Henriques Maria is grateful to acknowledge her PhD grant no. 2022.10965.BDANA awarded by Fundação para a Ciência e a Tecnologia.

P33 - Fluorescent Ureido-Dihomooxacalix[4]arene-Based Receptors for Nitroaromatic Compounds

Gil, Beatriz V. (1); Miranda, Alexandre S. (1); Marcos, Paula M. (1, 2); Ascenso, José R. (3); Berberan-Santos, Mário N. (4).

(1) – Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Edifício C8. (2) – Faculdade de Farmácia, Universidade de Lisboa. (3) – Centro de Química Estrutural - Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa. (4) – Institute for Bioengineering and Biosciences, IST, Universidade de Lisboa.

The detection of explosives is a major task in the fight against terrorism and in homeland security. Nitroaromatic compounds (NACs), such as trinitrotoluene (TNT), dinitrotoluene (DNT) and trinitrophenol (TNP), are common explosives used for military purposes and are the principal components of unexploded landmines. They are also considered environmental pollutants. Low-cost detection techniques, with high portability, high sensitivity and selectivity are needed for in-field analyte effective sensing. Luminescence-based methods fulfil these requirements.1 Lately, a broad range of fluorescent sensors for explosive monitoring have been developed based on calixarenes.2 Fluorophores like naphthalene, anthracene and pyrene are among the most incorporated in the calixarene framework, leading to potential fluorescent probes for NACs. In the course of our recent studies on anion binding by fluorescent homooxacalixarenes,3,4 we have investigated the recognition of nitroaromatic compounds by these receptors. This work reports the affinity of dihomooxacalix[4]arene derivatives 1 and 2, containing naphthylurea residues at the lower rim, towards selected NACs. Their affinity was determined by fluorescence and NMR spectroscopy.

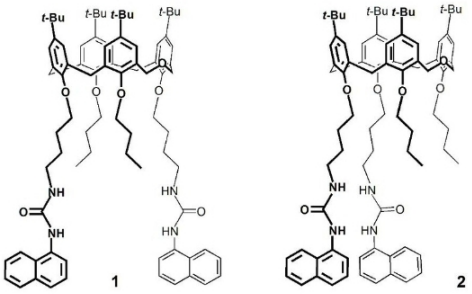


Figure 1: Chemical structures of the studied compounds 1 and 2

References

1 M. S. Meaney, V. L. McGuffin, Anal. Bioanal. Chem. 2008, 391, 2557-2576 2 R Kumar, A Sharma, H Singh, P Suating, H S Kim, K Sunwoo, I Shim, B C Gibb, J S Kim, Chem. Rev. 2019, 119, 9657-9721 3 A. S. Miranda, P. M. Marcos, J. R. Ascenso, M. N. Berberan-Santos, F. Menezes, Molecules 2022, 27, 3247

Acknowledgements

Acknowledgments: Authors thank Fundação para a Ciência e a Tecnologia, Projects UIDB/00100/2020, UIDP/00100/2020, LA/P/0056/2020 and UIDB/04565/2020.

P34 - Adhesive co-polymers for the development of electrochemical biosensing platforms

Carneiro, D. M. (1); Correia, J. P. (1); Viana, A. S. (1)

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

Enzymes are recognized for their catalytic activity and inherent selectivity to certain substrates, providing enzymatic biosensors high sensitivity to quantify analytes of interest, allowing to overcome the limitations of conventional analytical techniques (expensive equipment, long analysis times and the requirement of specialized technicians) [1]. In the case of an amperometric enzymatic biosensor, the developed interface must ensure robust immobilization of the enzyme and simultaneously allow the transduction of the electrochemical signal. Polydopamine (PDA) is a polycatecholamine with promising physical and chemical characteristics, however, PDA is poorly conductive [2], self-limiting its electrochemical growth and amperometric transduction efficiency. To overcome these limitations, this study aims to explore the electro-co-polymerization of dopamine and pyrrole, to take advantage of the catechol and amine groups of PDA – immobilization of the enzyme, combining them with the conductive character of polypyrrole (PPy) – electron transfer reactions. In this study, the co-polymers synthesis was achieved using two electrochemical modes – potentiodynamic and potentiostatic, and different proportions/concentrations of monomers were tested. The co-polymeric films were characterized by electrochemical, optical, spectroscopic techniques, and atomic force microscopy to assess their physicochemical properties. It was found that the combination of the two monomers has a synergistic effect on the growth of a conductive co-polymer that proves to be a suitable matrix for immobilizing enzymes (e.g. pyranose oxidase) with promising catalytic activity for glucose detection.

References

[1] Ispas, C. et al. Review: Recent Developments in Enzyme-Based Biosensors for Biomedical Analysis, Anal Lett, 2012, 45, 168 [2] Almeida, L. et al. Combined Electrochemical, Ellipsometric and Microgravimetric Study of Ion Permeable Polydopamine Films, J Electrochem Soc, 169, 046503

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

P35 - Impact of Hydroxylation Patterns of Very Long Chain Ceramides in Gel Phase Biophysical Properties

Çuhadar, E. (1); Bruyneel, M. (1); Marquês, J. T. (1); de Almeida, R. F. M. (1)

(1) - Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Ed.C8, Campo Grande, 1749-016 Lisboa, Portugal

Sphingolipids are deeply involved in numerous cellular functions, namely in the regulation of plasma membrane organization, particularly in fungi, where the existence of sphingolipid-enriched domains (SLEDs) in gel phase under physiological conditions is a trait not found in mammals [1]. Phytoceramides and ceramides, which differ in their hydroxylation levels, are the major backbone of complex sphingolipids in fungi and mammals, respectively [2]. In this study, we investigated the influence of different hydroxylation patterns in gel phase properties using five distinct very long chain ceramides. Lipid bilayers of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), an abundant phospholipid in the plasma membrane, and different molar proportions of each ceramide were prepared. These binary lipid bilayers were labeled with trans-parinaric acid (t-PnA), a fluorescent membrane probe particularly suitable to study gel domains, for which it presents a high partition coefficient and where it displays an increased quantum yield and a distinctive long fluorescence lifetime component. We found that the sphingolipid backbone hydroxylation profile had a significant impact on the hydrophobic chain packing in the gel phase. Partial phase diagrams obtained for the different POPC/(phyto)ceramide mixtures show that the thermal stability and sphingolipid-enrichment of the gel are also dependent on the hydroxylation pattern. Our investigation highlights the crucial role of hydroxylation in modulating gel phase biophysical properties, and therefore of SLEDs properties in the fungal plasma membrane, which are likely to influence the activity of several antifungal drugs.

References

[1] Santos, F. C. et al. FEBS Lett. 2020, 594, 3698-3718. [2] Marquês, J. T. et al. Prog. Lipid Res. 2018, 71, 18-42

Acknowledgements

This work was supported by Fundacao para a Ciencia e a Tecnologia (FCT), Portugal through CEECIND/03247/2018 to JTM, EXPL/BIA-BFS/1034/2021, Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

P36 - How closely do sphingolipids and sterols interact in yeast plasma membrane?

Savarino, R (1); Santos, F. C. (1); Gomes, M. (1); Coelho, B. (1); Leitão, H. (1); Guimarães, M. (1); Bento-Oliveira, A. (1); Marquês, J. T. (1); de Almeida, R. F. M. (1)

(1) - Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Ed.C8, Campo Grande, 1749-016 Lisboa, Portugal

With the worldwide increase in fungal infections and diminishing antifungal drug efficacy, fungal plasma membrane (PM) emerges as a potential target for enhanced antifungal therapies. Two major membrane compartments are present in yeast PM: MCC, occupied by Can1p and ergosterol-enriched and MCP, occupied by Pma1p and sphingolipid (SL)-enriched [1]. The presence of highly rigid SL-enriched domains (SLEDs) in S. cerevisiae PM under physiological conditions [2] is a major difference from the mammalian PM. The main goal of this work is to assess the impact of changing SL or sterol profile on yeast PM biophysical properties and organization using suspensions of intact cells and isolated PM labelled with fluorescent membrane probes. DPH, reporting on global membrane order, di-8-ANEPPS, which is particularly sensitive to sterol-enriched domains, reporting on properties such as membrane dipole potential and t-PnA, particularly suitable to study gel domains, were employed [2]. Membrane dipole potential is increased in erg6 cells compared to both wt and strains with altered SL profile. ERG6 deletion significantly affects sterol-rich domains, but not SLEDs. In turn, the hydrophobic packing in SLEDs is affected by SL profile, but not sterol. Unlike mammalian lipid rafts comprised of SLs and sterol, in yeast SLs and sterols segregate into different PM domains. The altered sensitivity to antifungals of the strains accumulating SLs different from the wt1 seems to correlate with the changes in membrane biophysical properties. Thus, the deeper understanding of the molecular basis explaining the main differences between fungal and mammalian membranes is crucial to overcome antifungal resistance [1].

References

[1] Santos, F. C. et al. FEBS Lett 2020, 594, 3698-3718. [2] Bento-Oliveira, A. et al. Biomol. 2020, 10, 871.

Acknowledgements

This work was supported by Fundacao para a Ciencia e a Tecnologia (FCT), Portugal through CEECIND/03247/2018 to JTM, EXPL/BIA-BFS/1034/2021, Centro de Química Estrutural is a Research Unit funded by FCT through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

P37 - Photoreduction of CO_2 with cryptates catalysts and visible light

Marques, R. T. (1); Realista, S. (1); Santos, R. G. (2); Martinho, P. N. (1)

(1) – Centro de Química Estrutural, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal. (2) – Centro de Recursos Naturais e Ambiente, Instituto Superior Técnico, Universidade de Lisboa, Portugal.

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)[1] or directly to liquid fuels (methanol and/or methane) [2]. The photoconversion of CO_2 can be made in homogeneous and heterogeneous media. The former has the advantadge of modulating the catalytic active sites to improve selectivity. It requires three components: the catalyst (CAT, which in the active form, converts CO_2), the sacrificial donor (SD, donates electrons and is consumed) and the photosensitiser (PS, absorbs light and mediates the electronic transfer between the CAT and the SD). Our research group reported Co(II)-cryptates with different substituents in the aromatic rings (-Br, $-NO_2$, -CCH) and observed that the capture and conversion of CO_2 were affected by them. The catalysts were able to produce CH_4 when higher irradiation times or higher amounts of catalyst were used [3]. We present the synthesis and characterisation of dinuclear cryptates with substituents in the aromatic ring. The photoreduction of CO_2 and the optimisation of the photocatalytic system and setup was also investigated.

References

[1] Sheng, W. et al. Energy Environ. Sci. 2017, 10, 1180. [2] Kuhl ,K. P. et al. J. Am. Chem. Soc. 2014, 136, 14107. [3] Realista, S. et al. Chem. Eur. J. 2019, 25, 11670.

Acknowledgements

Centro de Química Estrutural (CQE) and the Institute of Molecular Sciences (IMS) acknowledge financial support from Fundação para a Ciência e Tecnologia (Projects UIDB/00100/2020, UIDP/00100/2020, and LA/P/0056/2020, respectively. We are grateful to Fundação da Ciência e a Tecnologia, FCT, for Project PTDC/QUI-QIN/0252/2021 (https://doi.org/10.54499/PTDC/QUI-QIN/0252/2021). Paulo N. Martinho also thanks FCT for the contract CEECIND/00509/2017 (https://doi.org/10.54499/CEECIND/00509/2017/CP1387/CT0029). Sara Realista thanks FCT for the contract 2020.02134.CEECIND (https://doi.org/10.54499/2020.02134.CEECIND/CP1605/CT0002).

P38 - Magnetic MoO_2 Nanoparticles as Catalysts in the transformation of sulfur compounds

Moreira, P. (1); Henriques, A. C. (1); Nunes, C. D. (1)

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

The sulfur content in fuels has faced increasingly stringent restrictions to comply with standards (10-15 ppm) set by regulatory entities. These limitations arise due to the adverse effects of sulfur compounds on both human health and the environment. To address this, various techniques are employed, including oxidative desulfurization (ODS). ODS is particularly promising because of its mild operating conditions and high efficiency. Catalyst was prepared by supporting MoO_2 nanoparticles on Fe_3O_4 nanoparticles. After synthesis and characterization this catalyst was tested in the oxidation of four sulfides commonly found in fuels: diphenyl sulfide, methyl phenyl sulfide, 1-benzothiophene, and dibenzothiophene. Reactions were conducted at 80 ℃ using H_2O_2 and tert-butyl hydroperoxide as oxidants, with different substrate-oxidant ratios (1:1 or 1:2 mmol). The catalytic tests demonstrated excellent yields, especially for methyl phenyl and diphenyl sulfide. Additionally, the feasibility of catalyst recovery via magnetic separation was explored, suggesting practical applications.

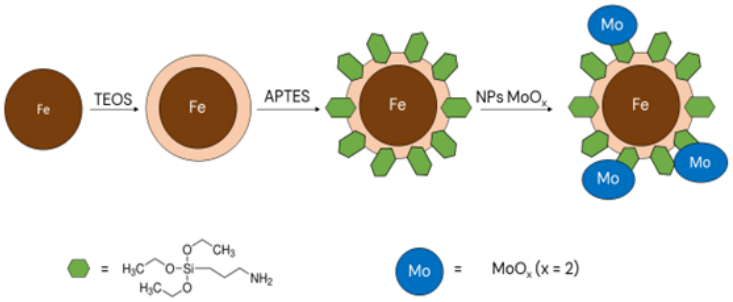


Figure 1: $MoO_2 - Fe$ nanoparticles catalyst synthesis

References

[1] Hossain, M.N. et al. Catalysts 2019, 9, 229. [2] Rajendran, A. et al. Mater. Chem. A. 2020, 8, 22462285.

Acknowledgements

This work was supported by Fundação para a Ciência e Tecnologia through project with DOI 10.54499/2022.03236.PTDC, Centro de Química Estrutural, by UIDB/00100/2020 and UIDP/00100/2020 and Institute of Molecular Sciences an Associate Laboratory funded by FCT through project LA/P/0056/2020.

Mini-posters

4th Chem & Biochem
Students Meeting
June 27, 2024
FCUL - Lisboa

M1 - Tau liquid-liquid phase separation

Carolino, I. (1)

Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

Emerging studies suggest that liquid-liquid phase separation (LLPS) is a crucial mechanism underlying the formation of membraneless organelles in eukaryotic cells. These structures are involved in numerous biological processes and, therefore, implicated in both human health and disease. Recent findings show that tau, whose aggregation plays a major role in Alzheimer's disease, has a high propensity to form liquid condensates through LLPS. In this review, the authors show that the driving forces of tau LLPS are dynamic and variable interactions, which can be favored or disturbed by binding of various molecules, post-translational modifications, and temperature variation. The implications of tau LLPS in tau aggregation, whose underlying mechanism is still largely misunderstood, are also discussed, with divergent opinions on whether tau LLPS is on pathway to amyloid aggregation or if these just occur in overlapping conditions.

References

[1] Li, P. et al. Liquid - liquid phase separation of tau: Driving forces, regulation, and biological implications. Neurobiol Dis. 2023, 183, 106167.

Acknowledgements

I thank everyone at the Protein Misfolding and Amyloids in Biomedicine laboratory at the Faculty of Sciences University of Lisbon for the support and encouragement.

M2 - Mechanisms of Action of Mu-Opioid Receptors in Opioid Dependency

Vitorino, D. (1)

(1) - Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

Opioids are one of the most effective treatment drugs for severe and chronic pain. Although in these cases its administration is necessary to provide relief, it causes a number of issues between tolerance, addiction and abuse [1]. Furthermore, the consequences of their illicit availability and use can often result in an opioid-related overdose, in many cases leading to death [2]. Therefore, understanding the mechanisms of action of the different opioid receptors becomes essential to help those that struggle with addiction. Over the years, there has been developing research about the binding to the GPCR mu-opioid receptor (MOR) in the central nervous system, and as well as possible treatment targets. This review focuses on MOR mechanism of action in dependency cases and potential therapies.

References

[1] Pergolizzi JV Jr, LeQuang JA, Berger GK, Raffa RB. The Basic Pharmacology of Opioids Informs the Opioid Discourse about Misuse and Abuse: A Review. Pain Ther. 2017 Jun;6(1):1-16. [2] Piyusha P. Pagare, Rachael Flammia, Yan Zhang, IUPHAR review: Recent progress in the development of Mu opioid receptor modulators to treat opioid use disorders, Pharmacological Research, 2024, V 199.

Acknowledgements

M3 - Monitoring trace levels of organophosphate pesticides in water: An HFμE/GC-MS(SIM) approach

Soares, M. (1); Nogueira, José M. F. (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

To enhance agricultural productivity various approaches have been employed such as the use of organophosphate pesticides. Although organophosphate pesticides exhibit lower persistence, concerns remain regarding their environmental impact. The current study proposes the utilization of hollow fiber microextraction in combination with gas chromatography-mass spectrometry for monitoring trace levels of five organophosphate pesticides (Ethoprophos, Diazinon, Methyl Parathion, Malathion and Chlorpyrifos) in water matrices. The primary objective is to develop, optimize, and validate an innovative and alternative methodology. This technique will be applied to water matrices of various types, including tap-water, wastewater, and irrigation water. The optimization stage analysis was conducted using a vaporization injector in the splitless mode. In the validation stage, a programmed temperature vaporization injector will be used to achieve lower detection limits in compliance with legal standards for pesticide determination in drinking water (0.1 μg/L for individual and 0.5 μg/L for total, respectively). During the microextraction stage optimization, we assayed several experimental parameters, including the selectivity of different organic solvents, equilibrium time, agitation speed, polarity and ionic strength. Additionally, we investigated the desorption time during the back-extraction stage. The optimized conditions, tested with six replicates, yielded average recoveries ranging from 31.9$\%$ to 88.8$\%$, with a relative standard deviation ranging from 0.16$\%$ to 6,78$\%$.

References

[1] Souza Pinheiro, A et al (2009). Talanta, 79(5), 1354–1359. [2] Ore, O. T et al (2023). J. Environ Chem and Ecotoxicol, 5, 9–23. [3] Ide, A. H et al (2020). Int J Environ Anal Chem, 100(12), 1402–1414.

Acknowledgements

Centro de Química Estrutural is a Research Unit funded by Fundação para a Ciência e a Tecnologia (FCT) through projects UIDB/00100/2020 (https://doi.org/10.54499/UIDB/00100/2020) and UIDP/00100/2020 (https://doi.org/10.54499/UIDP/00100/2020). Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020 (https://doi.org/10.54499/LA/P/0056/2020).

M4 - Phase Separation and Misfolding of TDP-43 in Neurodegenerative Diseases

Silva, C. M.

(1) - MSc in Biochemistry and Biomedicine - Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal; (2) - Intern at the Protein Misfolding and Amyloids in Biomedicine Laboratory - BioISI, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Transactive response DNA-binding protein 43 (TDP-43) is a nucleic acid-binding-protein with important physiological roles in RNA metabolism, regulating transcription and translation, mRNA splicing, transport and stabilization, non-coding RNA processing and stress granule (SG) assembly in neurons. TDP-43 has been implicated in a wide range of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Alzheimer's and Lewy bodies disease. It has been suggested that the mechanisms underlying TDP-43 neuropathology consist of nuclear loss-of-function and cytoplasmic gain-of-function through depletion of TDP-43 in the nucleus and mislocalization and further aggregation in the cytoplasm. TDP-43 undergoes liquid-liquid phase separation (LLPS) to form membraneless organelles, likely contributing to RNA processing spatiotemporal control in the nucleus. Moreover, upon transient cellular stress, the intrinsically disordered low complexity domain (LCD) of TDP-43 promotes LLPS mediating reversible assembly of SG in the cytoplasm which can become irreversible. In neurodegenerative diseases, mislocalization of TDP-43 enhances the transition of TDP-43 liquid droplets into a solid state leading to spontaneous aggregation and neurodegeneration. Furthermore, it has been evidenced that misfolded TDP-43 might propagate toxicity in a prion-like manner. This mini review aims to provide an overview of the current understanding of TDP-43's role in health and disease. I will discuss the complex mechanisms, including toxic self-assembly and phase separation, that contribute to neurodegeneration, and the existing knowledge gaps in this area.

References

[1] Liao, YZ. et al. Mol Neurobiol. 2022, 59, 4223–4241; [2] Chien, HM. et al. Int. J. Mol. Sci. 2021, 22:8213; [3] de Boer, EMJ. et al. J Neurol Neurosurg Psychiatry. 2020, 92, 86–95; [4] Keating SS. et al. Prog Neurobiol. 2022, 211:102229; [5] Carey, JL. Guo, L. Front Mol Biosci. 2022, 9:826719.

Acknowledgements

EU for funding Twinning Grant EU-TWIN2PIPSA/GA101079147, FCT/MCTES (Portugal) for funding UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/MULTI/04046/2020 (DOI: 10.54499/UIDP/04046/2020) (BioISI) and Agilebio for funding LabCollector Scientific Award 2021 (CMG).