P.PORTO

ESCOLA SUPERIOR DE SAÚDE

8th meeting on medicinal biotechnology

BOOK OF ABSTRACTS

MAY 29TH, 2026
SCHOOL OF HEALTH
POLYTECHNIC OF PORTO

8th meeting on
medicinal biotechnology

May 29th, 2026
School of Health
Polytechnic of Porto

8th meeting on medicinal biotechnology

BOOK OF ABSTRACTS OF THE 8TH MEETING ON MEDICINAL BIOTECHNOLOGY

School of Health - Polytechnic of Porto
Rua Dr. António Bernardino de Almeida, 400
4200 - 072, Porto
Portugal

ISBN: 978-989-9226-20-3
DOI: HTTPS://DOI.ORG/10.26537/ED.P.PORTO.251

May, 2026

8th meeting on medicinal biotechnology

May 29th, 2026
School of Health
Polytechnic of Porto

8th meeting on
medicinal biotechnology

SCIENTIFIC COMMITTEE

Cassilda Pereira
Clara Pereira
Cristina Prudêncio
João Rufo
Mariana Ferreira
Mónica Vieira
Pedro Coelho
Ricardo Ferraz
Tony Rodrigues
Vanda Peixoto
Virgínia Cruz Fernandes

ORGANIZING COMMITTEE

Afonso Silva
Beatriz Alves
Beatriz Bessa
Cassilda Pereira
Clara Pereira
Cristina Prudêncio
Dulce Teixeira
Inês Mestre
João Rufo
Maria Almeida
Maria Inês Caçoilo
Maria Teresa Amaral
Mariana Almada
Mariana Ferreira
Mónica Vieira
Pedro Coelho
Ricardo Ferraz
Rui Pedro Silva
Tony Rodrigues
Vanda Peixoto
Virgínia Cruz Fernandes

8th meeting on ***medicinal biotechnology***

PROGRAM

13:30	Welcome
14:00	Opening Session
14:20	**Plenary** "*We engineer nature's brilliance to pioneer a new approach to colour*" Miguel Garrido | Colorifix
15:20	Oral Communications I *"Biotechnological Potential of Macroalgae-associated Actinomycetota"* Gabriela Moreira ""*Skin Delivery of Polyphenols: Microencapsulation and Skin Equivalents in Cosmetic Innovation*" Mariana Marques "*Sustainable Purification of IgY Antibodies via Pear Peel-Based Graphene Aerogels to Tackle Antimicrobial Resistance*" Leonel Guerra
15:50	Coffee Break & Poster Session
16:30	**Plenary** "*Emerging microbial-based technologies for oral vaccination in aquaculture*" Cláudia Serra | CIIMAR
17:30	Oral Communications II "*Novel sustainable biomaterials coated with polydopamine*" João Nunes "*Functional biomembranes based on polysaccharides and polyphenols to improve oral mucositis*" Ana Carolina Alves "*Antioxidant and antimicrobial potential of green extracts made from coastal dune plants*" Kiano Gorissen
17:50	Closing Session & Awards

8th meeting on medicinal biotechnology

PLENARY SPEAKERS

We engineer nature's brilliance to pioneer a new approach to colour

MIGUEL GARRIDO COSTA[1]

1. COLORIFIX, CAMBRIDGE, UNITED KINGDOM (WWW.COLORIFIX.COM)

Miguel Garrido Costa has a career at the intersection of biotechnology and industrial sustainability, supported by a bachelor's and master's degree in Biochemistry and Biotechnology and, currently finishing a doctorate in Textile Chemical Engineering, with studies at UTAD, ESS-IPP and the Minho University. His professional career was initially consolidated at Archroma and, subsequently, over four years at RDD (Research Design and Development), where, as a Researcher and Scientific Coordinator, he led the implementation of sustainable innovations with pioneering global brands such as H&M, Pangaia and Inditex.

For the past five years at Colorifix, Miguel Garrido Costa has held the position of Implementation Lead, assuming strategic responsibility for coordinating the scale-up and global implementation of biological dyeing technology, establishing himself as a central figure in the transposition of laboratory science to the industrial scale and in the ecological modernization of the global textile industry.

Colorifix is at the forefront of innovation in the textile dyeing - but not only - industry, pioneering a biological process that produces, deposits and fixes pigments onto fabrics at a commercial scale. Unlike traditional dyeing methods, which rely heavily on toxic chemicals and consume vast amounts of water and energy, Colorifix uses engineered microbes to achieve the same results in a more sustainable and environmentally friendly way. By replacing inefficient chemical processes with biological alternatives, Colorifix addresses the significant environmental challenges posed by an industry that consumes over 5 trillion litres of water annually. The company has successfully created several biological colours that can be grown in custom bioreactors and a range of these colours is already available for use by brands. With strategic partnerships across the fashion industry, Colorifix is collaborating to replace conventional dyeing methods with its innovative technology. Their solution offers a scalable, environmentally responsible alternative to the harmful practices currently dominating textile dyeing.

Colorifix continues to advance its technology, it is well-positioned to drive a major shift toward sustainability in one of the world's most resource-intensive industries, such as Polymers, Cosmetics and varnish industries, making eco-conscious dyeing a reality for brands and consumers alike.

KEYWORDS: *colorifix*, *sustainability*, *biological dyes*, *fermentation*

Emerging microbial-based technologies for oral vaccination in aquaculture

CLÁUDIA R. SERRA[1,2]

1. CIIMAR – CENTRO INTERDISCIPLINAR DE INVESTIGAÇÃO MARINHA E AMBIENTAL
2. FCUP – FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO

Aquaculture is a fundamental pillar of the European Blue Bioeconomy, and its sustainable growth is essential for feeding the world's growing population. The occurrence of infectious diseases, however, represent a major constraint, casuing substantial economic losses globally. Vaccination is widely considered as the most appropriate method for pathogen control on economic, environmental, and ethical grounds.

Oral vaccines are highly demanded by the aquaculture sector as an alternative to expensive, labor-intensive injectable vaccines, which require individual fish handling and cause stress-related immunosuppression and mortalities. Despite this demand, most previous attempts to develop effective oral vaccines in fish have failed.

One promising strategy involves bacterial spores, particularly those of Bacillus subtilis, as injection-free vaccine delivery vehicles. Their extreme resistance ensures passage through the harsh gut environment without loss of function, making them well-suited for oral applications. Key advantages include direct incorporation into animal feed, simple production, and long shelf-life without refrigeration. Beyond this "needle-free" and "refrigeration-free" potential, B. subtilis spores also exhibit adjuvant properties and contribute to gut-associated lymphoid tissue (GALT) development, further enhancing their value as oral antigen delivery systems.

A second strategy involves bacterial extracellular vesicles (EVs), which are spherical, bilayered nanostructures released by the bacterial cell envelope. These naturally carry membrane proteins, lipids, and metabolites, making them attractive platforms for antigen delivery and vaccination.

The Microbiology and Biotechnology in Aquaculture (MBAqua) research group of CIIMAR is exploring both B. subtilis spores[1,4] and EVs from Gram-negative[2] and Gram-positive[3] bacteria as carriers of antigens from a range of problematic bacterial and viral pathogens. The group's latest results will be presented and discussed.

Keywords: *aquaculture, oral vaccines, bacterial spores, evs*

References:

1 Gonçalves G, Santos RA, Cerezo IM, Gabriel T, Dias J, Magalhães R, Pousão-Ferreira P, Enes P, Tafalla C, Oliva-Teles A, Couto A, Díaz-Rosales P and Serra CR (2026) Antigen-displaying probiotic Bacillus subtilis spores induce subtle, strain-dependent immunomodulation in fish. Front. Mar. Sci. 13:1774354. doi: 10.3389/fmars.2026.1774354

2 Matinha-Cardoso J, Gonçalves G, Coutinho F, Lima S, Bonneville L, Serrano M, Tamagnini P, Oliva-Teles A, Couto A, Serra CR, Oliveira P (2026). Cyanobacterial Extracellular Vesicles as Protein Carriers: Towards Fish Vaccination. Microb Biotechnol. 2026 Jan;19(1):e70294. doi: 10.1111/1751-7915.70294.

3 Vicente-Gil S, Nuñez-Ortiz N, Morel E, Serra CR, Docando F, Díaz-Rosales P, Tafalla C (2024). Immunomodulatory properties of Bacillus subtilis extracellular vesicles on rainbow trout intestinal cells and splenic leukocytes. Front Immunol. 2024 May 7;15:1394501. doi: 10.3389/fimmu.2024.1394501.

4 Gonçalves G, Santos RA, Coutinho F, Pedrosa N, Curado M, Machado M, Costas B, Bonneville L, Serrano M, Carvalho AP, Díaz-Rosales P, Oliva-Teles A, Couto A, Serra CR (2022). Oral vaccination of fish against vibriosis using spore-display technology. Front Immunol. 2022 Oct 13;13:1012301. doi: 10.3389/fimmu.2022.1012301.

8th meeting on medicinal biotechnology

ORAL COMMUNICATIONS

Biotechnological Potential of Macroalgae-associated Actinomycetota

GABRIELA ALVES MOREIRA[1,2,3,4,5], MARIANA GIRÃO[1], NÁDIA EUSÉBIO[2,6], MARTA V. MENDES[1,4] & MARIA DE FÁTIMA CARVALHO[1,4]

1. CIIMAR/CIMAR-LA– INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH, MATOSINHOS, PORTUGAL. 2. NGC – NEXT GENERATION CHEMISTRY, MATOSINHOS, PORTUGAL. 3. I3S - INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE, PORTO, PORTUGAL. 4. ICBAS - SCHOOL OF MEDICINE AND BIOMEDICAL SCIENCES, UNIVERSITY OF PORTO, PORTO, PORTUGAL. 5. E2S - POLYTECHNIC OF PORTO, PORTO, PORTUGAL. 6. KOD BIO, R. DO PROGRESSO 145, 4455-533 MATOSINHOS, PORTUGAL.

Actinomycetota are Gram-positive bacteria widely distributed across diverse environments, from terrestrial to marine ecosystems, where they may occur as free-living organisms or in association with other organisms. This phylum is well-known for its remarkable biotechnological potential, with several members producing bioactive compounds such as antimicrobial and anticancer agents. However, macroalgae-associated Actinomycetota remain underexplored despite having a metabolic potential comparable to free-living organisms.

The main objective of this study was to explore the biosynthetic potential of 15 Actinomycetota isolates obtained from two marine macroalgae from the Portuguese coast, *Codium tomentosum* and *Chondrus crispus*, through the analysis of their genomes.

Total DNA was sequenced using Illumina technology and 17 *de novo* genomes were assembled. Phylogenomic analysis identified 5 different genera, *Actinoalloteichus*, *Cellulosimicrobium*, *Kocuria*, *Nocardia* and *Streptomyces*, with 3 putative novel species.

Genome mining, using manually curated antiSMASH data, identified 452 biosynthetic gene clusters (BGCs) across 21 metabolite classes. Among the BGCs identified are antimicrobial and anticancer compounds, siderophores and conserved metabolites. More than 60% of these BGCs have none or low similarity to previously known compounds, which can be an indicator of novelty. Compared with *Streptomyces*, one of the most studied genera, non-*Streptomyces* genera exhibited a higher novelty percentage (ca. 80%).

Additionally, BiG-SCAPE and clinker were used to visualize the data and assess the similarity between BGCs for the same compound in different strains.

Overall, these results confirm that macroalgae-associated Actinomycetota represent a valuable and underexplored source of new species and biosynthetic diversity, supporting further study of this bacterial group.

KEYWORDS: *actinomycetota, novelty; biosynthetic potential*

ACKNOWLEDGMENTS:
This research was funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025) and UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025). G.A.M was supported by a FCT PhD grant (2023.04554.BDANA; DOI: 10.54499/2023.04554.BDANA). M.V.M. was supported by the FCT CEEC IND6ed funding program with reference 2023.08302.CEECIND/CP2848/CT0019 (DOI: 10.54499/2023.08302.CEECIND/CP2848/CT0019).

Skin Delivery of Polyphenols: Microencapsulation and Skin Equivalents in Cosmetic Innovation

MARIANA MARQUES[1,2], FILIPA TEIXEIRA[3,4], BERTA ESTEVINHO[5,6], STEFANO DALL'ACQUA[7] & FRANCISCA RODRIGUES[1,2]

1. UCIBIO, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 2. ASSOCIATE LABORATORY I4HB, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 3. REQUIMTE/LAQV, ISEP, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 4. ICBAS, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 5. LEPABE, FACULTY OF ENGINEERING, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 6. ALICE, FACULTY OF ENGINEERING, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 7. DEPARTMENT OF PHARMACEUTICAL AND PHARMACOLOGICAL SCIENCES, UNIVERSITY OF PADOVA, PADOVA, ITALY. * CORRESPONDENCE: FRODRIGUES@FF.UP.PT

Polyphenols are promising cosmetic active ingredients due to their antioxidant and skin-protective properties, but their application is limited by instability, poor solubility, and restricted permeation[1]. Microencapsulation in biocompatible polymers improves stability and provides prolonged release[1]. In parallel, the ban on animal testing under the European Cosmetic Regulation (CE) No. 1223/2009 has accelerated the need for advanced skin equivalents[2]. In this study, catechin, epicatechin, chlorogenic (CGA), neochlorogenic acid (NCGA), and a mixture of these polyphenols were encapsulated via spray-drying in sodium alginate. Microparticles were characterized by SEM, DSC, and FTIR, confirming the successful incorporation of the bioactives. Encapsulation significantly improved antiradical and antioxidant activities, with evidence of synergistic interactions in the mix. Biocompatibility in HaCaT keratinocytes and HDF fibroblasts showed that encapsulated polyphenols were better tolerated than their free forms. Skin delivery was assessed with an *in vitro* 3D skin equivalent model and compared with *ex vivo* explants as well as a commercial 3D model (SkinEthic™). Encapsulation enabled sustained release in the 3D model, while *ex vivo* studies displayed negligible permeation for flavan-3-ols and higher skin permeation and retention rates for CGA, NCGA, and the mixture (**Fig.1**). Overall, encapsulation enhanced the stability and biocompatibility of polyphenols, supporting their use in cosmetic formulations. The combined use of 3D and *ex vivo* models provided relevant insights into topical delivery.

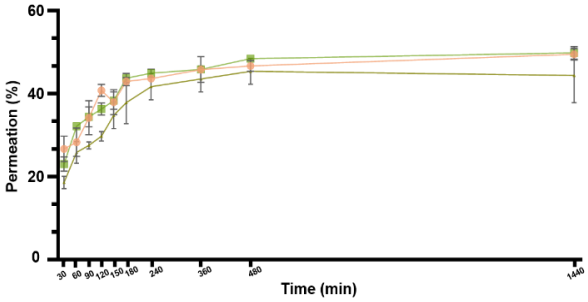

Fig 1. Release profile of CGA, NCGA and the mix at 500 mg/mL concentration in *ex vivo* skin explants using Franz diffusion cells and quantified by LC-DAD-MS. Results are expressed as mean ± standard deviation (SD) (*n*=3).

KEYWORDS: *3d skin model, permeation studies, polyphenols.*

ACKNOWLEDGMENTS/FUNDING:
This work was supported by FCT/MCTES (Portugal) through: FireSkin (2024.07134.CBM), UID/50006, UID/04378/2025, UID/PRR/04378/2025, LA/P/0140/2020, and individual grants (2024.01202.BD, 2023.14718.TENURE.016).

REFERENCES:
1Bińkowska, W.; Szpicer, A.; Stelmasiak, A.; Wojtasik-Kalinowska, I.; Półtorak, A. Appl. Sci. 2024, 14, 11954.
2Vinardell, M. P. Regul. Toxicol. Pharmacol. 2015, 71 (2), 198-204.

Sustainable Purification of IgY Antibodies via Pear Peel-Based Graphene Aerogels to Tackle Antimicrobial Resistance

LEONEL GUERRA[1], ANA CAROLINA PINTO[1], CATARINA ALMEIDA[1], KUMUD MALIKA TRIPATHI[2], EMANUEL V. CAPELA[1] & MARA G. FREIRE[1]

1. CICECO – AVEIRO INSTITUTE MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, AVEIRO, PORTUGAL 2. DEPARTEMENT OF BIONANOTECHNOLOGY, GACHON UNIVERSITY, 1342 SEONGNAM-DAERO, SUJEONG-GU, SEONGNAM-SI, GYEONGGI-DO 13120, SOUTH KOREA.

Recently, the inappropriate consumption of antibiotics contributed to the progression of Antimicrobial Resistance (AMR) scenario, with Methicillin-resistant Staphylococcus aureus (MRSA) standing out as one of the most dangerous bacteria [1]. Therefore, pursuing alternative therapeutics to the conventional antibiotics, namely biopharmaceuticals, is imperative. Avian Immunoglobulin Y (IgY), an antibody that could be recovered from hen egg yolk, is a promising solution. Owing to its polyclonal nature, IgY recognizes numerous epitopes of an antigen, minimizing the resistance [2].

Additionally, three-dimensional carbon nanostructures, such as carbon aerogels, are promising in the downstream processing of biopharmaceuticals, due to their versatile characteristics and inherent attractive properties, namely their sustainability and nontoxicity to humans [3].

Thus, in this work, the potential of a graphene aerogel obtained from pear peel was evaluated as an alternative low-cost approach for the downstream processing of IgY from egg yolk, for its potential application as a biopharmaceutical to tackle infectious diseases.

Based on this, the pear graphene aerogels were firstly characterized by FTIR, and their point of zero charge was determined. Then, the downstream process of IgY from the water-soluble protein fraction of egg yolk was developed and optimized, in terms of pH, contact time and solid:liquid ratio. Performance parameters such as process yield and purification factor were determined, and the protein profile was ascertained by SDS-PAGE. Under the optimal conditions, promising yields and purity values were obtained, surpassing those previously reported in the literature.

In summary, this approach allows to take full advantage of raw materials from nature, and the results reported herein represent a stepping stone to the application of sustainable pear graphene as highly efficient matrices for the downstream processing of value-added biopharmaceuticals from real matrices, such as IgY antibodies, helping to overcome the challenges faced due to AMR.

KEYWORDS: *antimicrobial resistance, immunoglobulin y (igy); graphene aerogels*

ACKNOWLEDGEMENTS:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). Emanuel V. Capela acknowledge FCT for the research contract 2024.08677.CEECIND.

REFERENCES:
1 Alghamdi, B. A.; Al-Johani, I.; Al-Shamrani, J. M.; Alshamrani, H. M.; Al-Otaibi, B. G.; Almazmomi, K.; Yusof, N. Y. Saudi J. Biol. Sci. 2023, 30, 103604.
2 Pereira, E. P. V.; Van Tilburg, M. F.; Florean, E. O. P. T.; Guedes, M. I. F. *Int. Immunopharmacol.* 2019, *73*, 293–303.
3 Myung, Y.; Jung, S.; Tung, T. T.; Tripathi, K. M.; Kim, T. *ACS Sustainable Chem. Eng.* 2019, *7*, 3772–3782.

Novel sustainable biomaterials coated with polydopamine

JOÃO C.F. NUNES, INÊS S.R. VIEIRA, MARA G. FREIRE, MÁRCIA C. NEVES & ANA P.M. TAVARES

1. CICECO – AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, 3810-193 AVEIRO, PORTUGAL

Sustainability is an opening new frontier in materials science. Hence, the development of eco-friendly materials functionalized with polydopamine, a versatile, bioinspired polymer derived from mussel adhesive proteins[1,2]efficient, simple and cost-effective separation of such pollutants from water is still challenging. Methods: A cellulose-based pH-responsive smart membrane with excellent reversible wettability was developed. Cellulose membrane was coated with polydopamine (PDA was investigated in this work. As a novel and sustainable approach, the polymerization of dopamine for materials coat was carried out enzymatically using laccase, an oxidoreductase enzyme that enables mild, aqueous, and catalyst-free conditions, eliminating the traditional need for harsh chemicals or metal catalysts[2]a bioinspired polymer from mussel adhesive proteins, has attracted impressive attention as a novel coating for (nano. This strategy significantly enhances the environmental process sustainability, while improving biocompatibility and preserving the integrity of materials surface and properties. Polydopamine-coated materials have several potential applications in medicinal biotechnology as wound dressings and biosensors. This work was focused on the optimization of natural materials coating with polydopamine to enhance biocompatibility, adhesion, and surface reactivity and the characterization of the novel sustainable coated biomaterials.

KEYWORDS: *sustainability; biomaterials; polydopamine; laccase.*

ACKNOWLEDGMENTS:
This work was financially supported by Fundação para a Ciência e Tecnologia, Exploratory Project 2024.16757.PEX. This work was funded by FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2030 in the framework of the project COMPETE2030-FEDER-00771100 (Refª FCT: 16538). This work was developed within the scope of the project CICECO Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC).

REFERENCES:
1Li, J.-W.; Wu, C.-M.; Chaudhuri, S. J. Taiwan Inst. Chem. Eng. 2026, 178, 106411.
2Magalhães, F.F.; Pereira, A.F.; Freire, M.G.; Tavares, A.P.M. Front. Bioeng. Biotechnol. 2022, 10, 1037322.

Functional biomembranes based on polysaccharides and polyphenols to improve oral mucositis

ANA CAROLINA ALVES,[1], JOANA GUEDES[2], LUÍS CRUZ[2] & SUSANA SOARES[1,2]

1. FCUP, DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY, FACULTY OF SCIENCES, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 2. REQUIMTE-LAQV, DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY, FACULTY OF SCIENCES, UNIVERSITY OF PORTO, PORTO, PORTUGAL.

Oral mucositis is a common and debilitating complication of radiotherapy and chemotherapy. This inflammatory condition is characterized by severe pain, erythema, and ulceration, leading to reduced oral intake, significant weight-loss, and deterioration in patients' quality of life [1]. As current therapeutic approaches rely mainly on basic oral care, anti-inflammatory agents, and pain management, more effective and accessible treatments are needed [2]. This study aims to develop functional biomembranes based on natural polysaccharides and plant-derived polyphenols with wound healing properties as a novel strategy for oral mucositis management.

The biocompatibility of various polysaccharides was evaluated using oral cell lines (HSC-3 and TR146) through MTT and Neutral Red assays. The selected polysaccharides were used to develop biomembranes by solvent casting method. Different families of plant-derived polyphenols (tannic acid, punicalagin, anthocyanins and procyanidins) were selected to enrich the biomembranes and several concentrations were assessed in cell viability assays.

Pullulan, sodium alginate, carboxymethylcellulose, pectin, banana and orange peel extracts showed moderate to high biocompatibility (60-120%) and were selected for further studies. Biopolymer solutions were optimized to determine the lowest concentration capable of forming the biomembranes (1% (w/v), 0.03g of polymer per 21.2 cm2 Petri dish). Further cell viability assays demonstrated that tannic acid maintained biocompatibility at lower concentrations (0.25, 0.5 and 1%), while higher concentrations resulted in a dose-dependent reduction in viability.

The selected polysaccharides demonstrated promising biocompatibility, indicating their potential as innovative alternatives for oral mucositis management. Ongoing studies are focused on further evaluating the biocompatibility of the polyphenols, as well as assessing the wound-healing performance of the functional biomembranes.

KEYWORDS: *oral mucositis, polysaccharides, polyphenols, biomembranes*

ACKNOWLEDGMENTS:
This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia, and Ministério da Educação, Ciência e Inovação) through the project UID/50006-Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.
Luís Cruz gratefully acknowledges FCT for the research contract funded through the Individual Call to Scientific Employment Stimulus with reference 2023.07923.CEECIND/CP2842/CT0001.

REFERENCES:
[1] Rodríguez-Caballero A, Torres-Lagares D, Robles-García M, Pachón-Ibáñez J, González-Padilla D, Gutiérrez-Pérez JL. Cancer treatment-induced oral mucositis: A critical review. Int J Oral Maxillofac Surg 2012;41:225–38. https://doi.org/10.1016/j.ijom.2011.10.011.
[2] Elad S, Cheng KKF, Lalla R V, Yarom N, Hong C, Logan RM, et al. MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. Cancer 2020;126:4423–31. https://doi.org/https://doi.org/10.1002/cncr.33100.

Antioxidant and antimicrobial potential of green extracts made from coastal dune plants

KIANO GORISSEN[1], AURORA SILVA[1,2], CLARA GROSSO & CRISTINA DELERUE-MATOS[1]

1. REQUIMTE/LAQV, INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO, INSTITUTO POLITÉCNICO DO PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, 431, 4249-015 PORTO, PORTUGAL 2. INSTITUTO DE AGROECOLOXÍA E ALIMENTACIÓN (IAA), INIVERSIDADE DE VIGO, NUTRITION AND FOOD GROUP (NUFOG), CAMPUS AUGA, 32004 OURENSE, SPAIN

Coastal dune plants have seen an increase in interest in the medicinal field, due to the identification of numerous bioactive compounds[1]. The Portuguese coast hosts a variety of plants, some of which have already shown high potential against oxidative stress[2]. However, traditional extraction techniques generally make use of organic solvents harmful to the environment or human health. Alternative extracts prepared from dune plants following the principles of green chemistry[3] remain understudied. This study aims to increase the current knowledge on antioxidant and antimicrobial potential of green extracts made from coastal dune plants. The coastal dune plants *Carpobrutus edulis* (L.) N.E. Br, *Corema album* (L.) D.Don and *Helichrysum italicum* (Roth) G.Don were collected from the Parque das dunas da Aguda. After drying by lyophilisation, the ash, lipid and protein contents of the plants were determined. Green extracts were produced from the dried plant matrices utilising subcritical water extraction and ultrasound-assisted extraction under multiple extraction conditions. Phenolic content and antioxidant activity of the extracts were determined by TPC method and by $DPPH^{\cdot}$, $ABTS^{\cdot+}$, $^{\cdot}NO$ and $^{\cdot}OH$ scavenging assays. Currently, the extracts with the highest scavenging potential will be selected for antimicrobial screening.

KEYWORDS: *dune coastal plants, antimicrobial activity; antioxidant activity*

ACKNOWLEDGMENTS:
This work received financial support from project "NUTRIBRAIN - Coastal Flora for Mental Well-being Promotion: Development of food supplements based on dune plant compounds with impact on BDNF-TrkB signalling" (COMPETE2030-FEDER-00686600, operation nº 15759), supported by FEDER (COMPETE 2030) and national funds by FCT / MECI. The authors also thanks the support of PT national funds (FCT/MECI) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. K. G. thanks FCT/MCTES for the financial support through a PhD fellowship (2024.16226.PRT). C. G. is thankful for her contract (2020.03436.CEECIND/CP1596/CT0008, DOI 10.54499/2020.03436.CEECIND/CP1596/CT0008) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.

REFERENCES:
1 Narayanan, M. *et al.* (2025). Coastal Sand Dune Plants as a Valuable Resource of Bioactive Metabolites in Pharmaceutical Field. In: Mérillon, JM., Ramawat, K.G. (eds) Plant Specialized Metabolites. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-031-51158-5_55
2Andrade, S.C., Guiné, R.P.F. & Gonçalves, F.J.A. Evaluation of phenolic compounds, antioxidant activity and bioaccessibility in white crowberry *(Corema album)* . *Food Measure* **11**, 1936–1946 (2017).
https://doi.org/10.1007/s11694-017-9576-43Agawa, T.; Miller, S. I. Am. Chem. Soc. 1961, 83, 449–453.
3 Chemat F, Vian MA, Cravotto G. Green extraction of natural products: concept and principles. Int J Mol Sci. 2012;13(7):8615-8627. doi: 10.3390/ijms13078615. Epub 2012 Jul 11. PMID: 22942724; PMCID: PMC3430255.

8th meeting on medicinal biotechnology

POSTER COMMUNICATIONS

Pan-Genomic and Thermodynamic Pipeline for the Design of Highly Specific Anti-Salmonella enterica Antisense Oligonucleotides

EMÍLIO GOMES[1,2], RICARDO J. FIGUEIREDO[3], DANIELA ARAÚJO[1], NUNO GUIMARÃES[2], NUNO F. AZEVEDO[2], JOANA CASTRO [1,4]

1.NATIONAL INSTITUTE FOR AGRARIAN AND VETERINARY RESEARCH (INIAV), VILA DO CONDE, PORTUGAL; 2 LEPABE – LABORATORY FOR PROCESS ENGINEERING, ENVIRONMENT, BIOTECHNOLOGY AND ENERGY, ALICE – ASSOCIATE LABORATORY IN CHEMICAL ENGINEERING, FACULTY OF ENGINEERING, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 3. SCHOOL OF MEDICINE AND BIOMEDICAL SCIENCES (ICBAS), UNIVERSIDADE DO PORTO (UP), PORTO, PORTUGAL; 4. CENTRE OF BIOLOGICAL ENGINEERING, UNIVERSITY OF MINHO, BRAGA, PORTUGAL.

Antimicrobial resistance in Salmonella enterica poses a serious threat to global public health and the poultry industry, underscoring the need for precision therapeutics to replace conventional broad-spectrum antibiotics [1]. Antisense oligonucleotides (ASOs) represent a promising targeted approach to control infectious diseases [2], however their success depends on careful target selection to ensure efficacy and rigorous off-target exclusion to avoid host dysbiosis. In this study, we developed a bioinformatic pipeline to select specific Salmonella targets to design highly specific anti-Salmonella ASOs. Following structural and biological quality control of 100 S. enterica genomes of *Gallus gallus* available from public databases, 91 high-quality assemblies were used to construct a robust pangenome. Core genes were screened for essentiality and virulence, followed by homology exclusion against avian commensal microbiota, closely related Enterobacteriaceae, and host genome. This pipeline identified pan-conserved, highly specific sequences targeting critical pathways, including LPS biosynthesis (rfaP) and flagellar motility (flhD). Selected genes were further analyzed to identify highly conserved 15- and 20-bp regions as candidate targets. These sequences underwent comprehensive off-target filtering against an extensive database of over 60,000 sequences (≤1 mismatch rejection), alongside strict biophysical evaluation to ensure purely linear secondary structures (ΔG=0.0 kcal/mol) at the mean avian physiological temperature of 42°C. Thermodynamic profiling indicated that 20-mer sequences exhibit significantly greater binding stability than 15-mers. Target mRNA accessibility was modeled to identify optimal candidates using a multiparametric decision matrix prioritizing strong hybridization affinity and minimal structural unfolding costs [3]. Furthermore, functional interaction networks validated the systemic impact of targets, while candidates were simultaneously screened for immunogenic liabilities as poly-G tracts, to prevent intermolecular aggregates. To enhance in vivo nuclease resistance and target affinity, the best-performing sequences were chemically modified using Locked Nucleic Acid (LNA) modifications [4]. Overall, this integrated strategy delivers robust, host-safe in silico candidates for further in vitro validation.

KEYWORDS: *antisense oligonucleotides; salmonella enterica; poultry; pangenomics; antimicrobial resistance; in silico drug design*

ACKNOWLEDGMENTS:
This work was financially supported by the NAMsal project 2022.07654.PTDC (https://doi.org/10.54499/2022.07654.PTDC) funded by national funds through the FCT/MCTES (PIDDAC). This study was also supported by FCT under the scope of the strategic funding of UIDB/04469/2020, UIDB/04050/2020 units and Oligos4Resistance project (https://doi.org/10.54499/2023.16872.ICDT), LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/ UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/ P/0045/2020). E.G. thanks FCT for the PhD Grant (2025.02246.BDANA). J.C. and D.A. thank FCT for Tenure contracts (2023.12090.TENURE.026 and 2023.12090.TENURE.028, respectively

REFERENCES:
1 Kumar, R.; et al. Sci. One Health 2025, 4, 100117.
2 Moustafa, D. A.; et al. FEMS Microbiol. Rev. 2026, 50.
3 Crooke, S. et al. Cell Metab. 2018, 27, 714–739.
4 Hagedorn, P. H.; et al. Drug Discov. Today 2018, 23, 101–114

Structure-Based Discovery of Novel Rabies Virus RdRp Inhibitors Using Molecular Dynamics Refinement and Virtual Screening of the NCI Diversity set

DUANGNAPA KIRIWAN[1], KIATTAWEE CHOOWONGKOMON[2] & SERGIO F. SOUSA[1,3]

1. LAQV/REQUIMTE, BIOSIM, DEPARTMENT OF BIOMEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 2. DEPARTMENT OF BIOCHEMISTRY, FACULTY OF SCIENCE, KASETSART UNIVERSITY, BANGKOK, THAILAND; 3. INSTITUTION, CITY, COUNTRY. CERES, BIA LAB, DEPARTMENT OF CHEMICAL ENGINEERING, FCTUC, UNIVERSITY OF COIMBRA, COIMBRA, PORTUGAL.

Rabies is a severe viral infection that causes brain inflammation[1]. It is transmitted from animals to humans through bites, licks or scratches on broken skin or mucosa. Once clinical symptoms, the disease is almost 100% fatal. Currently, post-exposure prophylaxis (PEP) remains the only effective intervention. The rabies virus (RABV) is an enveloped, single-stranded, negative-sense RNA virus belonging to the *Rhabdoviridae* family. The viral RNA-dependent RNA virus (RdRp) is a critical component for viral replication and transcription, making it a primary target for antiviral drug development. However, the 3D X-ray structure of the rabies lyssavirus RdRp protein has not yet been determined. In this study, we aimed to model the RABV RdRp protein to characterize its structural behavior. Using primary sequences from the NCBI databases, we employed structure prediction techniques, including sequence alignment, homology modeling, and refinement via molecular dynamics (MD) simulation clustering. Potential binding pockets were identified using three different algorithms the FTsite server[2], RaptorX[3], and CASTp[4] servers. Virtual screening was then performed against 2045 compounds from the NCI diversity dataset III using the GOLD CCDC docking program[5]. We identified the top five compounds with docking scores in the range from 70-90 kcal/mol. These candidates were further examined using binding modes and MM-PBSA binding free energy calculations. Our result indicate that compound name Z01690699 exhibited the most favorable binding score, forming stable hydrogen bonds and hydrophobic interactions within the catalytic site. These findings provide a structural foundation for the development of novel inhibitors targeting the rabies virus RdRp.

KEYWORDS: *rabies 1*, *rna-dependent rna polymerase 2*, *molecular modeling 3*, *virtual screening 4*, *molecular dynamics simulation 5*.

ACKNOWLEDGEMENTS:
This work was carried out within the support of the CERES Centre and supported by national funds from FCT through the projects https://doi.org/10.54499/UID/00102/2025 and https://doi.org/10.54499/UID/PRR/00102/2025. This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and 2024.00762.BD.

REFERENCES:
1 Fooks, A. R.; Cliquet, F.; Finke, S.; Freuling, C.; Hemachudha, T.; Mani, R. S.; Müller, T.; Nadin-Davis, S.; Picard-Meyer, E.; Wilde, H.; Banyard, A. C., Rabies. *Nature Reviews Disease Primers* **2017,** *3* (1), 17091.
2 Ngan, C.-H.; Hall, D. R.; Zerbe, B.; Grove, L. E.; Kozakov, D.; Vajda, S., FTSite: high accuracy detection of ligand binding sites on unbound protein structures. *Bioinformatics* **2011,** *28* (2), 286-287.
3 Kallberg, M.; Wang, H.; Wang, S.; Peng, J.; Wang, Z.; Lu, H.; Xu, J., Template-based protein structure modeling using the RaptorX web server. *Nat Protoc* **2012,** *7* (8), 1511-22.
4 Binkowski, T. A.; Naghibzadeh, S.; Liang, J., CASTp: Computed Atlas of Surface Topography of proteins. *Nucleic Acids Res* **2003,** *31* (13), 3352-5.
5 Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D., Improved protein-ligand docking using GOLD. *Proteins* **2003,** *52* (4), 609-23.

Mechanistic insights into HIV-1 intasome interaction with human nucleosomes

FÁBIO G. MARTINS[1], SÉRGIO F. SOUSA[1,2]

1. LAQV/REQUIMTE, BIOSIM DEPARTMENT OF BIOMEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, ALAMEDA PROFESSOR HERNÂNI MONTEIRO, PORTO, PORTUGAL 2. CERES, BIA LAB, DEPARTMENT OF CHEMICAL ENGINEERING, FCTUC, UNIVERSITY OF COIMBRA, RUA SÍLVIO LIMA, PÓLO II – PINHAL DE MARROCOS, 3030-790 COIMBRA, PORTUGAL

A key step in the HIV-1 life cycle is the integration of the viral genome into host chromatin, a process mediated by the intasome. While the intasome is known to target specific chromatin regions, the detailed molecular basis of how it interacts with the nucleosome, the basic unit of chromatin, has yet to be fully elucidated. In this study, we applied structure-based computational approaches, including molecular docking and molecular dynamics (MD) simulations, to investigate the interaction between the HIV-1 intasome and the human nucleosome. Initial models were based on crystal structures of the HIV-1 intasome and nucleosome (PDB IDs: 9C9M and 1KX5), followed by docking using HADDOCK 2.4. Subsequent MD simulations performed with Amber24 indicated that histone H4 tails and specific regions of histone H3 play a major role in stabilizing the complex. Additionally, our analysis highlighted the 51–56 region of nucleosomal DNA as an important contact site. Experimental evidence indicates that, unlike the PFV intasome, the HIV-1 intasome does not integrate efficiently across all nucleosomes within a trinucleosome. To investigate this, we carried out comparative docking studies using structures of the HIV-1 (9C9M) and PFV (3S3M) intasomes, and a human trinucleosome (6L4A). Our results suggest that PFV intasome can access all integration sites, whereas steric constraints limit the accessibility of the HIV-1 intasome.

KEYWORDS: *hiv, nucleosome; computational biochemistry.*

ACKNOWLEDGEMENTS:
This work was carried out within the support of the CERES Centre and supported by national funds from FCT through the projects https://doi.org/10.54499/UID/00102/ 2025 and https://doi.org/10.54499/UID/PRR/00102/2025. This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. We acknowledge the Academy of Finland for awarding this project access to the LUMI supercomputer, owned by the EuroHPC Joint Undertaking, hosted by CSC (Finland) and the LUMI consortium through the Academy of Finland funding programme"

REFERENCES:
1.Wilson, M. D. et al. Nat. Commun. 10, 4189 (2019).
2.Li, M. et al. Viruses 16, 1166 (2024).
3.Davey, C. A., Sargent, D. F., Luger, K., Maeder, A. W. & Richmond, T. J. J. Mol. Biol. 319, 1097–1113 (2002).
4.Takizawa, Y. et al. Structure 28, 44-53.e4 (2020).

Evaluation of Deep Learning Models for Aptamer Modelling In Silico

FÉLIX DIAS[1,3], FÁBIO MARTINS[3] & SÉRGIO F. SOUSA[2,3]

1. ESS, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 2. CERES, BIA LAB, DEPARTMENT OF CHEMICAL ENGINEERING, FCTUC, UNIVERSITY OF COIMBRA, RUA SÍLVIO LIMA, PÓLO II – PINHAL DE MARROCOS, 3030-790 COIMBRA, PORTUGAL; 3. LAQV/REQUIMTE, BIOSIM, DEPARTMENT OF BIOMEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, ALAMEDA PROF. HERNÂNI MONTEIRO, 4200-319 PORTO, PORTUGAL

Aptamers are single-stranded DNA or RNA oligonucleotides that fold into complex three-dimensional structures to bind molecular targets with high affinity and specificity (1). Often termed "chemical antibodies," these biomaterials are vital for diverse applications in therapeutics, diagnostics, bioassays, and in vitro and in vivo imaging (2). Historically, aptamer discovery has relied on the Systematic Evolution of Ligands by EXponential Enrichment (SELEX), an in vitro process involving iterative selection, amplification and enrichment rounds. However, SELEX is time-consuming and often results in low success rates (3). Consequently, an *in silico* approach is essential to streamline the discovery process, reduce experimental costs, and provide insights into aptamer-target interactions. Recently, various deep learning models capable of predicting the 3d structure of aptamer-target complexes have become available. In this work, we evaluated general biomolecular prediction models, like Alphafold-3, Protenix-v2, Chai-1, Boltz-2, Openfold-3, RoseTTAFold2NA, and two specialized aptamer modelling tools, AptaTrans and AptaBLE. Among the evaluated models, AlphaFold-3 stands as the current state-of-the-art general model for aptamer modelling, with Protenix-v2, Chai-1, Boltz-2 and RoseTTAFold2NA, accuracies falling short of AlphaFold-3. The only model that rivals Alphafold-3 in aptamer-target benchmarks is OpenFold-3. On the other hand, specialized tools such as AptaTrans and AptaBLE, significantly outperform AlphaFold3 on validated DNA and RNA aptamers, which can be attributed to their optimization for sequence-based patterns. Overall, this work highlights the importance of an *in silico* approach for aptamer discovery and evaluates deep learning models capable of predicting the 3D structure of aptamer-target complexes. While general models provide a robust baseline for aptamer-target complex structure prediction, specialized tools offer enhanced performance for aptamer discovery. Future work will include a systematic comparison of the evaluated models.

KEYWORDS: *aptamers*, *deep learning*, *in silico*

REFERENCES:

1Zhang Y, Lai BS, Juhas M. Recent Advances in Aptamer Discovery and Applications. Molecules. 2019;24(5):941. doi:10.3390/molecules24050941 PubMed PMID: 30866536.

2Sun H, Zu Y. A Highlight of Recent Advances in Aptamer Technology and Its Application. Molecules 2015, Vol 20, Pages 11959-11980. 2015 Jun 29;20(7):11959–80. doi:10.3390/molecules200711959 PubMed PMID: 26133761.

3Zhuo Z, Yu Y, Wang M, Li J, Zhang Z, Liu J, et al. Recent Advances in SELEX Technology and Aptamer Applications in Biomedicine. Int J Mol Sci. 2017 Oct 14;18(10). doi:10.3390/ijms18102142 PubMed PMID: 29036890.

Hybrid Computational Modelling of Human Voltage-Gated Sodium Channels for Isoform-Specific Therapeutic Design

JOÃO PEDRO BOAZINHA[1], JOÃO CARNEIRO[2], NUNO M.F.S.A. CERQUEIRA[1] & SÉRGIO F. SOUSA[1, 3]

1. LAQV/REQUIMTE – BIOSIM – DEPARTMENT OF BIOMEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 2. UNIVERSIDADE CATÓLICA PORTUGUESA, CBQF - CENTRO DE BIOTECNOLOGIA E QUÍMICA FINA – LABORATÓRIO ASSOCIADO, ESCOLA SUPERIOR DE BIOTECNOLOGIA, PORTO, PORTUGAL; 3. CERES, BIA LAB, DEPARTMENT OF CHEMICAL ENGINEERING, FCTUC, UNIVERSITY OF COIMBRA, COIMBRA, PORTUGAL.

Voltage-gated sodium channels (NaV) are membrane proteins that shift between open and closed conformations when the membrane voltage changes, allowing Na^+ to pass through excitable cells such as neurons and muscle fibers. This function is fundamental for action potential initiation and propagation, supporting neuronal activity, muscle contraction, and electrical communication throughout the body[1, 2].In humans, NaV isoforms show distinct tissue distributions and biophysical features that define their roles. NaV1.1, NaV1.2, NaV1.3, and NaV1.6 are mainly associated with central nervous system excitability, NaV1.4 regulates skeletal muscle contraction, NaV1.5 drives cardiac action potentials, and NaV1.7, NaV1.8, and NaV1.9 are involved in peripheral pain transmission. Because of this diversity, achieving isoform selectivity remains both a major challenge and a major opportunity in drug discovery[1, 2]. A complete atomic-level view of human NaV channels is still lacking, since experimental X-ray and cryo-EM structures are limited, unavailable for many isoforms, or structurally incomplete. This work presents a hybrid modelling strategy that combines artificial intelligence and physics-based refinement to build realistic 3D models of NaV channels in a human-like cytoplasmic environment. The workflow includes: (1) generating the five best full-length models with AlphaFold3, including auxiliary subunits; (2) comparing these models with structures obtained from other AI-based predictors, such as AlphaFold2, Chai-1, and Boltz-2, as well as partial experimental structures from the Protein Data Bank, using RMSD; (3) manually correcting loop regions in PyMOL to resolve topological issues; (4) constructing a realistic heterogeneous membrane with Packmol-Memgen using lipid compositions representative of human cells; and (5) refining the final systems by molecular dynamics simulations with Lipid17, Lipid21, and ff14SB force fields. These findings reveal structural differences among NaV isoforms that may guide the design of more selective compounds and reduce off-target effects.

KEYWORDS: *voltage-gated sodium channel, ai-tools, nav, alphafold*

ACKNOWLEDGEMENTS:
This work was carried out within the support of the CERES Centre and supported by national funds from FCT through the projects https://doi.org/10.54499/UID/00102/ 2025 and https://doi.org/10.54499/UID/PRR/00102/2025. This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and FCT (2023.04909.BDANA)

REFERENCES:
1Pan. *et al.* Science. 2018, 362(6412), p. eaau2486.
2Li, Z., Chen, L. e Li, H. Current Medical Science. 2019, 39(6), 863–873.

IHC-Based Identification of Molecular Targets in Canine Oncology: Towards Precision Veterinary Medicine

RITA FILES[1]*, JUSTINA PRADA[1,2]; FILIPE SILVA[1,2], AND ISABEL PIRES[1,2]

1 DEPARTMENT OF VETERINARY SCIENCES, UNIVERSITY OF TRÁS-OS-MONTES AND ALTO DOURO, 5000-801 VILA REAL, PORTUGAL; 2 ANIMAL AND VETERINARY RESEARCH CENTRE (CECAV), ASSOCIATE LABORATORY FOR ANIMAL AND VETERINARY SCIENCES (AL4ANIMALS), UNIVERSITY OF TRÁS-OS-MONTES AND ALTO DOURO, 5000-801 VILA REAL, PORTUGAL *ANAFILES@UTAD.PT

Neoplasms are a leading cause of morbidity and mortality in dogs, representing an increasing challenge in veterinary clinical practice. The morphological and biological diversity of canine tumors demands precise diagnostic approaches that complement conventional histopathology and provide clinically and prognostically relevant information. In this context, immunohistochemistry (IHC) plays a central role in veterinary oncology. It enables the identification of tumor cellular origin and histological classification, while also allowing the evaluation of molecular markers associated with tumor behaviour and the identification of potential therapeutic targets. Proteins such as Ki-67, E-cadherin, estrogen and progesterone receptors, HER-2, and VEGF have been extensively studied in canine tumors, showing consistent associations with histological grade, proliferative activity, invasiveness, and survival. The identification of therapeutic targets through IHC, including growth factor receptors, anti-apoptotic proteins, and oncogenic signalling pathways, has contributed to the development of targeted therapies in veterinary oncology, aligning it more closely with advances in human medicine. This is particularly relevant in aggressive tumors with poor prognosis, such as high-grade mammary carcinomas, mast cell tumors, and lymphomas, where molecular characterization can guide more precise and individualized treatment strategies. A notable example is the canine cutaneous histiocytoma, a benign tumor that undergoes spontaneous regression mediated by cytotoxic T lymphocytes. Its immunohistochemical characterization provides valuable insights into tumor–immune system interactions and represents a useful model for studying antitumor immune responses. This review aims to summarize current knowledge on the application of IHC in canine tumors, emphasizing its prognostic value, therapeutic relevance, and limitations, as well as highlighting the dog as a valuable model for comparative oncology within the One Health framework.

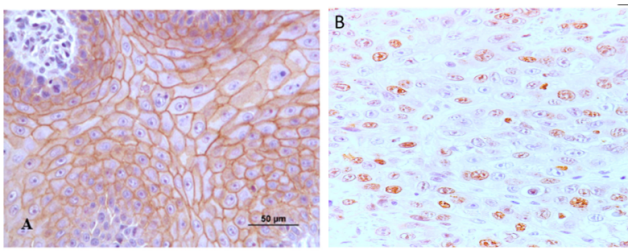

Figure 1: Different expression patterns of two biomarkers, E-cadherin and Ki-67 in canine squamous cell carcinoma. **A – E-cadherin:** Strong membranous labeling (intensity score 3) in a well-differentiated tumor (Grade I). **B – Ki-67:** High proliferation index in a moderately differentiated tumor (Grade III), (400×).

KEYWORDS: *immunohistochemistry, therapeutic target, precision medicine, one health*

FUNDING:
This work was carried out within the scope of the AICanceR∞Hub project (NORTE2030-FEDER-02687800), co-financed by the European Regional Development Fund (ERDF) under the Northern Regional Program 2021-2027 [NORTE2030].

REFERENCES:
Dos Anjos, D., et al (2021). . Life, 11(12), 1321. https://doi.org/10.3390/life11121321
Files, R., et al (2024). Veterinary Sciences, 11(12), 652. https://doi.org/10.3390/vetsci11120652
Luís, J., et al (2024). Current Issues in Molecular Biology, 46(5), 4951–4967. https://doi.org/10.3390/cimb46050297
Parisi, F., et al (2024). Animals, 14(10), 1400. https://doi.org/10.3390/ani14101400

Glioma behavior under the influence of Cinnamic Acid: a cellular approach

ANA MOREIRA[1,2], JOANA ALMEIDA[1] & RICARDO FERRAZ[1,2,3]

1. CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY RESEARCH (TBIO)/HEALTH RESEARCH AND INNOVATION (RISE–HEALTH), POLYTECHNIC INSTITUTE OF PORTO, SCHOOL OF HEALTH, PORTO, PORTUGAL; 2. SCHOOL OF HEALTH (E2S), POLYTECHNIC INSTITUTE OF PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200-072, PORTO, PORTUGAL; 3. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO, PORTO, PORTUGAL.

Background: Cancer is one of the biggest health problems worldwide, characterized by uncontrolled proliferation, rapid growth and the ability to invade other tissues (1). Gliomas are highly challenging tumours from a therapeutic perspective, so new and more effective therapeutic strategies have been investigated, with particular focus on the therapeutic potential of plant-derived compounds, such as Cinnamic Acid (2,3). Cinnamic Acid, extracted from *Cinnamomum cassia*, has proved to be a promising therapeutic agent, as it exhibits antitumor properties and low cytotoxicity toward healthy cells (4–6). This phenolic compound has the ability to cross the blood-brain barrier and affect tumour cell survival by reducing their proliferation rate, inducing apoptosis and inhibiting key signalling pathways involved in tumour growth (7–9).

Methods: This study aims to evaluate the glioma behaviour under the influence of cinnamic acid, using a GL261 cell model. To this end, cells were cultured and treated with different concentrations of cinnamic acid ($1x10^{-3}$ mol/L to $4x10^{-3}$ mol/L), followed by in vitro assays to assess its impact on cell viability and cell migration.

Results: The MTT assay performed on the GL261 cell line demonstrated that Cinnamic Acid is capable of reducing cell viability at all tested concentrations, with a cytotoxic effect that tends to be dose-dependent. At lower concentrations ($1x10^{-3}$ mol/L and $2x10^{-3}$ mol/L), cell viability was approximately 83% (SD=13,3% and 13,9%, respectively) and the most pronounced reduction in cell viability was detected at the highest tested concentration ($4x10^{-3}$ mol/L), with a viability of 76% ± 13,9%, corresponding to an approximately 24% reduction relative to the control (viability of 100%).

KEYWORDS: *glioma*, *cinnamic acid*, *antitumoral effects*, *therapeutic potential*, *cell culture*, *mtt assay*

REFERENCES:

1Linares CA, Varghese A, Ghose A, Shinde SD, Adeleke S, Sanchez E, et al. . International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI);2023;24(17):13215.

2Pellerino A, Caccese M, Padovan M, Cerretti G, Lombardi G. Clinical and Translational Imaging. Springer Science and Business Media Deutschland GmbH; 2022. p. 467–475.

3Kampers LFC, Metselaar DS, Vinci M, Scirocchi F, Veldhuijzen van Zanten S, Eyrich M, et al. Multidisciplinary Digital Publishing Institute (MDPI); 2025.

4Ruwizhi N, Aderibigbe BA. International Journal of Molecular Sciences. MDPI AG; 2020; 21(16): 1–36.

5Feng LS, Cheng JB, Su WQ, Li HZ, Xiao T, Chen DA, et al. Archiv der Pharmazie. John Wiley and Sons Inc; 2022;355(7).

6Niero ELDO, MacHado-Santelli GM. Journal of Experimental and Clinical Cancer Research. 2013;32(1).

7Lee AG, Kang S, Im S, Pak YK. Pharmaceutics. 2022;14(8).

8Meirelles LEF, Souza MVF, Carobeli LR, Morelli F, Mari NL, Damke E, et al. Biomedicines. Multidisciplinary Digital Publishing Institute (MDPI); 2023;11(2):275.

9Kim YN, Patil K, Pai SB. BMC Res Notes. 2025 Dec 1;18(1).

Fluoroquinolone-Based RNA Riboswitch Binders as Novel Drug Discovery Platforms Against Antimicrobial Resistance

ÂNGELA DO Ó[1], MANUELA MACHADO[2], EDUARDO SOARES[2,3,4], JORGE GARRIDO[5], ALEXANDRA GASPAR[1]

1. CIQUP-IMS, DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY, FACULTY OF SCIENCES, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 2. CIETI-BIOENGINEERING LABORATORY, ISEP, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 3. CEB-CENTRE OF BIOLOGICAL ENGINEERING, UNIVERSITY OF MINHO, BRAGA, PORTUGAL; 4. LABBELS-ASSOCIATE LABORATORY, BRAGA/GUIMARÃES, PORTUGAL; 5. CIQUP-IMS, ISEP, POLYTECHNIC OF PORTO, PORTO, PORTUGAL.

Antimicrobial resistance (AMR) is a major global challenge, as antibiotics used in therapy are progressively losing effectiveness against bacterial infections. This poses a high health burden, reinforcing the urgent need for innovative antibacterial agents with novel mechanisms of action (MoAs)[1,2] and first-in-class compounds. One strategy to address this demand could be the discovery of new small molecules targeting unexplored antibacterial drug targets, such as the non-coding RNA structures known as riboswitches. Accordingly, this work aims to discover and develop new RNA riboswitch ligands far from their cognate ones[3,4]. This work focuses on attaining a small library of fluoroquinolone-based compounds, to take advantage of their well-known antibacterial properties[5]. Ciprofloxacin, a representative fluoroquinolone antibiotic (Figure 1), was used as the starting scaffold for the design of new derivatives. Heterocyclic fragments containing sulfur, oxygen, and nitrogen, previously described as RNA ligands, were incorporated into the fluoroquinolone core, as illustrated in Figure 1. The rationale for these modifications is to confer dual-target activity by introducing RNA riboswitch-binding capability alongside DNA gyrase/topoisomerase inhibition, thereby moving beyond the traditional MoAs towards first-in-class RNA riboswitch ligands and dual-target antibiotics. To date, a small library of rationally designed derivatives has been successfully synthesised and is undergoing phenotypic evaluation of antibacterial activity against Gram-positive and Gram-negative bacteria via minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays Several compounds show promising antibacterial activity, representing a potential advance in the fight against AMR.

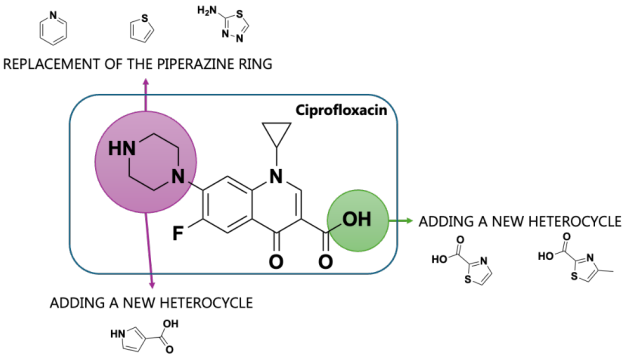

Figure 1: Rational Design of new antibacterials based on ciprofloxacin scaffold

KEYWORDS: *antimicrobial resistance (amr); rna riboswitch; ciprofloxacin; heterocycles; dual-target activity*

ACKNOWLEDGMENTS:
This work was FCT-funded–under project UIDB/00081/2025 (CIQUP) and LA/P/0056/2020 (IMS), DOI: 10.54499/LA/P/0056/2020.

REFERENCES:
1Ahmed, S. K., et al (2024). *J Med Surg Public Health*, 2, 100081. https://doi.org/10.1016/j.glmedi.2024.100081
2Mestrovic, T., et al (2025). *The Lancet Public Health*. https://doi.org/10.1016/S2468-2667(25)00201-4
3di Giorgio, A., & Duca, M. (2019). *MedChemComm*, 10(8), 1242–1255. https://doi.org/10.1039/C9MD00195F
4Serral, F., et al (2021). *Front.Pharmacol.*, 12, 647060. https://doi.org/10.3389/fphar.2021.647060
5Yanshu Jia, Liyan Zhao (2021). Eur. J. Med. Chem., 224, 113741. https://doi.org/10.1016/j.ejmech.2021.113741

Removal of dsRNA from In Vitro transcribed mRNA using multi-stage Aqueous Biphasic Systems

FILIPA DOMINGUES, MARIA I. SOUSA, AUGUSTO Q. PEDRO & MARA G. FREIRE

CICECO – AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, PORTUGAL.

Messenger RNA (mRNA) vaccines have demonstrated promising clinical outcomes in the prophylaxis of infections, capturing the interest of the scientific community and relevant stakeholders in the healthcare industry [1,2]. However, the conventional mRNA production process, which involves *in vitro* transcription (IVT), results in the generation of the byproduct double-stranded RNA (dsRNA)[3]. This contaminant drastically reduces mRNA translation efficiency *in vitro*, ex vivo, and for in vivo applications, making imperative its efficient removal. Currently, mRNA purification, including dsRNA removal, relies on multi-step procedures that involve chromatographic operations, enzymatic digestion, and filtration. Therefore, reducing process complexity and the number of unit operations requires developing an effective clarification step that removes dsRNA prior to chromatography[3]. In this context, aqueous biphasic systems (ABS), typically formed by mixing two water-soluble components at concentrations above specific thresholds, represent a promising alternative[4]. To enhance the separation performance, this work employs a multi-stage ABS strategy using polymer/polysaccharide compositions towards more economical and scalable processes. In this method, fresh top phases are sequentially introduced at each stage, promoting stepwise partitioning and progressive enrichment of the target molecule and dsRNA removal, thereby improving selectivity and purity. Initially, mRNA production by IVT was performed using a cell-free system with T7 polymerase. Next, phase diagrams were determined for selected ABS. The separation performance of single-stage and multi-stage systems was evaluated using agarose gel electrophoresis and dot-blot analysis. The results demonstrated that dsRNA preferentially partitions to the bottom phase of the system, whilst the target mRNA is enriched in the top phase, enabling its effective separation. In conclusion, this work demonstrates the potential of a multi-stage ABS-based approach as an efficient, scalable, and cost-effective strategy for dsRNA removal, contributing to the current mRNA purification process.

KEYWORDS: *mrna purification; double-stranded rna removal; multi-stage aqueous biphasic system; in vitro transcription.*

ACKNOWLEDGMENTS:
This work was developed within the scope of the project CICECO – Aveiro Institute of Materials, UID/50011/2025 & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). Augusto Q. Pedro acknowledges FCT for the research contract CEECIND/02599/2020 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) under the Scientific Stimulus–Individual Call. Maria I. Sousa acknowledges FCT for the PhD grant 2022.11476.BD.

REFERENCES:
1Gote, V. *et al.* International Journal of Molecular Sciences. 2023, 24 (3), 2700.
2LEE, K. *et al*. Nano Research. 2018, 11, 10, 5173–5192
3NOWAK, C. J. A. *et al*. Molecular Biology Reports. 2026, 53, 1.
4Iqbal, M. *et al.* Biological Procedures Online. 2016, 18, 18.

Enhancing the solubility and stability of pediatric tuberculosis first-line drugs using green solvents

MARIA INÊS CAÇOILO, VANESSA VIEIRA, EMANUEL V. CAPELA, MARA G. FREIRE

CICECO – AVEIRO INSTITUTE OF MATERIALS, UNIVERSITY OF AVEIRO, PORTUGAL

Tuberculosis (TB) remains one of the biggest causes of death globally with pediatric TB being a major concern due mainly to a lack of efficient pediatric formulations. Among the first line drugs used in this context, isoniazid is widely used in the management of TB across patient populations, irrespective of socioeconomic background, age group, or stage of therapy due to its bactericidal properties and relatively low toxicity[1]. However, the long regimen protocols currently in usage were developed for adults. The protocols used for pediatric treatment can lead to inaccurate dosing, reduced availability of the Active Pharmaceutical Ingredient (API) for absorption, impaired stability, and irregular bioavailability, which may compromise the treatment outcome and contribute to the development of drug resistance[2]. Deep Eutectic Solvents (DES) and Therapeutic Deep Eutectic Solvents (THEDES), have been explored as novel approaches to improve solubility, stability and biopharmaceutical performance of drugs like analgesics, anti-inflammatories and antifungals due to their compositional flexibility that tailor to the specific requirements of each medication, ultimately improving bioavailability and therapeutic outcomes[3]. In this context, we herein propose the use of green solvents to enhance the solubility and stability of anti-TB drugs with the intent of developing a new formulation that is more acceptable for pediatric use. Firstly, an HPLC (reversed-phase C18 column) method for isoniazid was developed and validated. Then, a wide plethora of DES were prepared and screened towards their potential to increase the solubility and stability of isoniazid. The API solubility was determined using the excess-solid method, with DES/THEDES at different molar ratios and water contents, and at 25 °C and 37 °C. Based on the obtained results, it was found that the HBA and HBD chemical structures play an important role in the formation of DES, and in the cases where the formation of DES occurs, they can, indeed, improve the solubility of isoniazid. These results provide new insights into DES influence on the solubility/stability of anti-TB drugs, proving their capability to deeply impact the biomedical field and the global human health.

KEYWORDS: *tuberculosis, isoniazid, deep eutectic solvents*

ACKNOWLEDGMENTS:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). Emanuel V. Capela acknowledge FCT for the research contract 2024.08677.CEECIND.

REFERENCES:
1 Adeleke, O. A., Hayeshi, R. K., & Davids, H. (2020). Development and evaluation of a reconstitutable dry suspension containing isoniazid for flexible pediatric dosing. Pharmaceutics, 12(3), 286.
2 Court, R., Chirehwa, M. T., Wiesner, L., de Vries, N., Harding, J., Gumbo, T., ... & McIlleron, H. (2019). Effect of tablet crushing on drug exposure in the treatment of multidrug-resistant tuberculosis. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease, 23(10), 1068.
3 Abdelquader, M. M., Li, S., Andrews, G. P., & Jones, D. S. (2023). Therapeutic deep eutectic solvents: A comprehensive review of their thermodynamics, microstructure and drug delivery applications. European Journal of Pharmaceutics and Biopharmaceutics, 186, 85-104.

Impact of Simulated Digestion on Microplastics: Surface Changes and Intestinal In Vitro Exposure

MARIANA LAMAS[1,2], VIRGÍNIA CRUZ FERNANDES[1,3], MARTA OLIVEIRA[1] & FRANCISCA RODRIGUES[4,5]

1. REQUIMTE/LAQV, ISEP, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 2. ICBAS - SCHOOL OF MEDICINE AND BIOMEDICAL SCIENCES, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 3. RISE-HEALTH, CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY RESEARCH (TBIO), ESS, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 4. I4HB – ASSOCIATE LABORATORY INSTITUTE FOR HEALTH AND BIOECONOMY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 5. UCIBIO – APPLIED MOLECULAR BIOSCIENCES UNIT, MEDTECH-LABORATORY OF PHARMACEUTICAL TECHNOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL.

Microplastics (MP) have been widely detected in a variety of food products[1], and ingestion is one of the main pathways for human exposure to these contaminants. Once ingested, MP might interact with the gastrointestinal (GI) environment, yet it remains unclear whether digestive processes alter their chemical structure or mainly influence their surface properties, and possibly their interaction capacity[3]. This study investigates digested-induced modifications in MP and establishes suitable conditions for intestinal *in vitro* assays. Simulated digestion of six polymers (PTFE; PMMA, PA6, uPVC, PET, and LDPE) followed the INFOGEST model[2]. Following digestion, particles were analysed using Fourier-transform infrared spectroscopy (FTIR) to assess structural and surface changes. Cell viability was evaluated, by MTT, in Caco-2 and HT29-MTX intestinal cell lines exposed to different dilutions (1:10, 1:20, and 1:40) of digestion fluids, with respective MP. FTIR results indicated that all polymers maintained their fundamental chemical structure after digestion. Instead, consistent surface-related alterations were observed, including reduced intensity of characteristic polymer bands and enhanced signals in regions associated with proteins and hydroxyl groups, suggesting biomolecules adsorption and increased hydration. Cell studies demonstrated that 1:20 dilution of digestive fluids with MP maintained the cell viability above 70%, representing the most suitable condition tested. Simulated GI digestion appears to preserve the intrinsic chemical identity of MP, while promoting surface modifications that may affect their biological interactions. The identification of 1:20 digestion fluid dilution supports its application in future studies using advanced intestinal models, including three-dimensional systems and assessment of cellular oxidative stress responses.

KEYWORDS: *gastrointestinal digestion, chemical structure; intestinal cell models*

FUNDING:
This work received financial support from the PT national funds (FCT/MECI) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and RISE-Health, R&D Unit (reference UID/06397/2025), and from the FEDER - the European Regional Development Fund (COMPETE 2030), through the WISE project - "Improving water for a safer environment" (No. 17091, ref. COMPETE2030-FEDER-00841800). M. Lamas (2024.05133.BD) would like to thank FCT/MECI and ESF for her PhD grant. M. Oliveira and F. Rodrigues thank to their TENURE programs (PRR No. 02/C06-i06/2024 and 2023.14718.TENURE.016, respectively).

REFERENCES:
1Giri S, et al. Microplastics contamination in food products: occurrence, analytical techniques and potential impacts on human health. Curr Res Biotechnol. 2024;7:100190.
2Minekus M, et al. A standardised static in vitro digestion method suitable for food—an international consensus. Food Funct. 2014;5:1113–1124.
3Prata JC, et al. Environmental exposure to microplastics: an overview on possible human health effects. Sci Total Environ. 2020;702:134455.

Profiling airway microbiome composition through volatilomics

FILIPA NETO[1,2], RICARDO FERRAZ[1,2,3], MÓNICA VIEIRA[1,2], CRISTINA PRUDÊNCIO[1,2] & JOÃO RUFO[1,2]

1. ESS, POLYTECHNIC OF PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA 400, 4200-072 PORTO, PORTUGAL. 2. RISE-HEALTH, CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA (TBIO), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL 3. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO, PORTO, PORTUGAL

The airway microbiome is known to mediate respiratory health. However, current available methods for microbiome analysis are time-consuming and/or represent significant costs for a generalized application in clinical practice. Volatilomics has been suggested as a rapid and low-cost approach to screen microbial profiles in human samples. Therefore, we aimed to study the efficacy of volatilomics in discriminating microbial isolates collected from human breath condensate samples. Bacterial strains showing significant growth under conditions simulating the respiratory environment were isolated. Each strain was standardised to an inoculum of 10^8 CFU/mL and analysed using an electronic nose equipped with a six-sensor matrix. Data were explored through principal component analysis, cluster analysis, pattern analysis, random forests and recursive partitioning regression. One sensor was removed from the analysis due to high collinearity. Principal component analysis was able to separate strains and the control mainly through the second principal component (p = 0.024), characterized by high MQ3 and MQ8 sensor responses. Sensor profile maps showed distinct volatile patterns across strains (Figure 1), suggesting the presence of distinct microbial signatures. However, reproducibility was low between replicas and time since culture. Recursive partitioning for separating sterile controls from inoculated samples showed the highest accuracy (AUC = 0.73). These results show the potential of separation of microbial strains based on volatilomics. Nonetheless, relative robustness was only achieved for the discrimination of sterile vs inoculated samples.

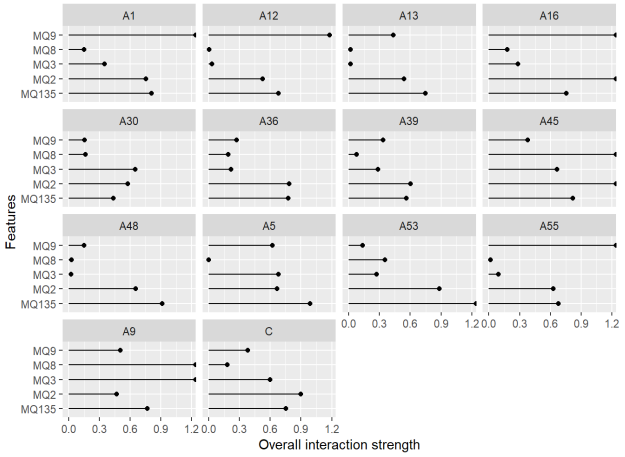

Figure 1 – Sensor profiles for the identified strains (A1 to A55) and controls (C).

KEYWORDS: *electronic nose*, *metabolomics*, *microbiome*, *volatile organic compounds*

FUNDING:
This work is funded by Fundação para a Ciência e Tecnologia (FCT) under grant agreement 2024.15890.PEX (DOI: https://doi.org/10.54499/2024.15890.PEX).

Enhancing Cosmetic Peptide Skin Permeation Using Ionic Liquids

BRUNA OLIVEIRA[1,2,3], MARIANA MARQUES[1,2], ANA GOMES[3], FRANCISCA RODRIGUES[1,2] PAULA GOMES[3],

1. I4HB – ASSOCIATE LABORATORY INSTITUTE FOR HEALTH AND BIOECONOMY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL 2. UCIBIO – APPLIED MOLECULAR BIOSCIENCES UNIT, MEDTECH-LABORATORY OF PHARMACEUTICAL TECHNOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL 3. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, FACULDADE DE CIÊNCIAS, UNIVERSIDADE DO PORTO, PORTO PORTUGAL

Skin has emerged as a versatile route for local and systemic drug delivery[1], but cutaneous pathologies such as psoriasis, atopic dermatitis, and wounds impair barrier function and alter skin structural integrity, which can compromise drug delivery[2]. In this context, ionic liquids (ILs) have emerged as promising third-generation permeation enhancers and a green alternative to conventional enhancers, offering tunable physicochemical properties that facilitate interaction with stratum corneum lipids, improving drug solubility and facilitating dermal and transdermal delivery, particularly for challenging molecules such as peptides[3]. Pentapeptide-4 (PP4) is a matrikine widely used in the cosmetic industry, known for its ability to stimulate collagen synthesis and support skin regeneration. When combined with imidazolium-based ILs, PP4-IL constructs, exhibit antimicrobial activity, provided by the ionic liquid, in addition to their tissue-rejuvenating properties, making this combination of interest for wound repair and inflammatory skin diseases, where enhanced local retention of bioactive peptides may be beneficial without systemic exposure[3, 4]. Therefore, this work investigates the ability of ILs to enhance PP4 retention in human skin, rather than to drive deep systemic permeation. Permeation assays were performed using Franz diffusion cells with human skin, and mass spectrometry to quantify retention of PP4-IL, PP4 control, and a hydrogel formulation containing the PP4-IL conjugate. The study will provide insights into the role of ILs in optimizing local accumulation of peptide-based actives, within human skin, for wound repair and chronic inflammatory skin conditions.

KEYWORDS: *dermal drug delivery; ionic liquids; pentapeptide-4; permeation enhancers; skin structure*

FUNDING:
Francisca Rodrigues and Ana Gomes (AG) were supported by FCT - Fundação para a Ciência e a Tecnologia, I.P under the TENURE program (2023.14718.TENURE.016 and 2023.14741.TENURE.005/CP00058/CT00008). The authors acknowledge the support from the Aliança para a Investigação e Ensino Superior, and the funding received by FCT I.P., in the scope of the project UIDP/04378/2020 (DOI:10.54499/UIDP/04378/2020) and UIDB/04378/2020 (DOI:10.54499/UIDB/04378/2020) of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI:10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. AG also thanks FCT for the project 2024.15574.PEX.

REFERENCES:
1 Lee, D. H. et al. Advancements in Skin-Mediated Drug Delivery: Mechanisms, Techniques, and Applications. Advanced Healthcare Materials 2024, 13 (7). DOI: 10.1002/adhm.202302375 (accessed 2025-10-22T14:00:15).
2 Montero-Vilchez, T. et al. Skin Barrier Function in Psoriasis and Atopic Dermatitis: Transepidermal Water Loss and Temperature as Useful Tools to Assess Disease Severity. Journal of Clinical Medicine 2021, 10 (2), 359. DOI: 10.3390/jcm10020359 (accessed 2026-01-02T13:48:08).
3 Gomes, A. et al. The Emerging Role of Ionic Liquid-Based Approaches for Enhanced Skin Permeation of Bioactive Molecules: A Snapshot of the Past Couple of Years. International Journal of Molecular Sciences 2021, 22 (21), 11991. DOI: 10.3390/ijms222111991 (accessed 2025-10-30T12:50:39).
4 Gomes, A. et al. Boosting Cosmeceutical Peptides: Coupling Imidazolium-Based Ionic Liquids to Pentapeptide-4 Originates New Leads with Antimicrobial and Collagenesis-Inducing Activities. Microbiology Spectrum 2022, 10 (4). DOI: 10.1128/spectrum.02291-21 (accessed 2025-10-21T13:00:42).

Integrating mRNA production and clarification using thermoreversible aqueous biphasic systems and ionic liquids

MARIA I. SOUSA, MARA G. FREIRE, FRANCISCA A. E SILVA & AUGUSTO Q. PEDRO

CICECO – AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, PORTUGAL

Despite the potential of messenger RNA (mRNA) vaccines in the prophylaxis and therapeutics of human diseases, mRNA nanomedicine production is still a complex and expensive process that requires improved technologies to produce more stable and widely accessible products, meeting a timely and sufficient manufacturing capacity [1]. Aqueous biphasic systems (ABS) are liquid–liquid systems formed by mixing aqueous solutions of different components above critical concentrations and are widely applied in biomolecule purification [2]. Ionic liquids (ILs), organic salts with highly tuneable structures, have emerged as promising additives due to their ability to enhance RNA stability [3] and improve ABS selectivity [4]. Here, we report for the first time the integration of *in vitro* transcription (IVT) of mRNA within an ABS environment containing 2% (w/w) IL. This unprecedented approach enables the simultaneous production and initial clarification of mRNA in a single platform. mRNA partitioning and integrity were assessed by electrophoresis and analytical chromatography, while the levels of double-stranded RNA (dsRNA) contaminants were evaluated via dot-blot analysis. The results demonstrate that IVT can proceed efficiently in the presence of ABS components and ILs, yielding stable mRNA with competitive production levels. Notably, the hydrophilic character of the ILs influenced transcription performance, with certain systems enhancing mRNA yield. Furthermore, selected ABS formulations showed promising capacity for dsRNA removal, supporting their role in downstream clarification. Overall, this work introduces a novel, integrated production–clarification strategy based on IL-based thermoreversible ABS. By performing IVT directly within a phase-separating system, this approach reduces process complexity, lowers costs and environmental impact, and improves mRNA stability and manufacturing efficiency, offering a compelling alternative for next-generation mRNA vaccine production.

KEYWORDS: *mrna production, in vitro transcription; process integration; aqueous biphasic systems; ionic liquids*

ACKNOWLEDGMENTS:
This work was supported by FCT – Fundação para a Ciência e Tecnologia, I.P. by project reference 2022.11476.BD and DOI identifier 10.54499/2022.11476.BD. This work was developed within the scope of the project CICECO – Aveiro Institute of Materials, UID/50011/2025 & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). Augusto Q. Pedro and Francisca A. e Silva acknowledge FCT respectively, for the research contracts CEEC-IND/02599/2020 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) and CEEC-IND/03076/2018 under the Scientific Stimulus–Individual Call. Maria I. Sousa acknowledges FCT for the PhD grant 2022.11476.BD.

REFERENCES:
1Pardi N., Hogan M.J., Porter F.W., Weissman, D. Nature Reviews Drug Discovery. 2018, 17, 261-279.
2 Pereira J. F. B., Freire M. G., Coutinho J. A. P. Fluid Phase Equilibria. 2020, 505, 112341.
3 Pedro A.Q., Pereira P., Quental M.J., Carvalho A.P., Santos S.M., Queiroz J.A., Sousa F., Freire M.G. ACS Sustainable Chemistry & Engineering. 2018, 6, 16645-16656.
4 Ventura S.P.M., e Silva F.A., Quental M.V., Mondal D., Freire M.G., Coutinho J.A.P. Chemical Reviews. 2017, 117, 6984-7052.

Ethnobotany and Bioactive Compounds: Bridging Traditional Knowledge and Biotechnology

AIRAM ROGGERO[1]*, AMAURI ALVES[2], YOANNIS DOMÍNGUEZ RODRÍGUEZ[3], MARCOS H. TOYAMA[4], SERGIO F. SOUSA[1,5]

1. LAQV/REQUIMTE — BIOSIM, DEPARTMENT OF BIOMEDICINE, MEDICINE FACULTY OF PORTO UNIVERSITY; 2. EPVC - ESCOLA PROFISSIONAL DE VILA DO CONDE - ASSOCIAÇÃO COMERCIAL E INDUSTRIAL DE VILA DO CONDE, PORTUGAL; 3. DEPARTMENT OF BIOLOGY, INSTITUTE OF BIOSCIENCES UNESP/CLP, BRAZIL; 4. BIOMOLPEP, DEPARTMENT OF BIOLOGY, INSTITUTE OF BIOSCIENCES UNESP/CLP, BRAZIL; 5. CERES, BIA LAB, DEPARTMENT OF CHEMICAL ENGINEERING, FCTUC, UNIVERSITY OF COIMBRA (*)AIRAM.ROGGERO@HOTMAIL.COM

Medicinal plants represent an important source of bioactive compounds with potential pharmacological and biotechnological applications. Natural products have historically played a central role in drug discovery, highlighting the importance of ethnobotanical knowledge as a valuable guide for identifying biologically active molecules. However, this traditional knowledge is increasingly threatened by cultural transitions and the loss of intergenerational knowledge transmission. This work presents the Ethnobotanical EPVC Project, developed at the Escola Profissional de Vila do Conde as an interdisciplinary initiative within the vocational program in Sociocultural Animation. The project integrates vocational education, academic research institutions and local communities with the aim of documenting traditional medicinal plant uses, promoting scientific literacy among students and exploring the phytochemical potential of plant species with possible therapeutic relevance. Emphasis is given to PANCs (non-conventional food plants), which represent an important yet underexplored component of local biodiversity with potential nutritional, medicinal and biotechnological applications. The methodological framework includes ethnobotanical surveys, botanical identification, ecological observation and preparation of plant-derived extracts. Students participate in field activities; community interviews and the documentation of traditional medicinal practices associated with these species. Selected plants are further explored through preliminary phytochemical perspectives and the development of natural formulations such as plant extracts, herbal preparations and essential oil-based products. The project benefits from collaboration with researchers from Universidade do Porto and the Universidade Estadual Paulista, enabling the integration of traditional knowledge with modern scientific approaches including phytochemistry, molecular biology and computational studies of bioactive compounds. This interdisciplinary initiative demonstrates how vocational education can contribute to early-stage bioprospecting and scientific engagement. It also highlights the potential of ethnobotanical knowledge and underutilized plant species as a bridge between community heritage, education and medicinal biotechnology, promoting biodiversity awareness and the sustainable exploration of plant-derived bioactive compounds.

KEYWORDS: *ethnobotany; medicinal plants; bioactive compounds; traditional knowledge; science education.*

ACKNOWLEDGEMENTS:
This Research thanks FCT (Fundação para a Ciência e Tecnologia) for PhD grant ref. BD.2023.00566. and received support from CERES Centre and supported by national funds from FCT through the projects https://doi.org/10.54499/UID/00102/ 2025 and https://doi.org/10.54499/UID/PRR/00102/2025.This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.. para a Química Verde - Tecnologias e Processos Limpos.

Enhancing Antifungal Activity Against Candida albicans Using Novel Organic Compounds

BEATRIZ ALVES[1,3], RUI SILVA[1], MÓNICA VIEIRA[1], RICARDO FERRAZ[1,2], SÍLVIA FERNANDES[1], VITOR TEIXEIRA[3] & CLARA PEREIRA[1]

1. RISE-HEALTH, CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY RESEARCH (TBIO), ESS, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL; 2. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO, PORTO, PORTUGAL; 3. I3S, INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE, IBMC, INSTITUTO DE BIOLOGIA CELULAR E MOLECULAR, UNIVERSIDADE DO PORTO, PORTO, PORTUGAL.

Candida albicans is the predominant cause of invasive candidiasis, a major global health threat [1 The increasing emergence of resistance to commonly used antifungals (ex.:iazoles and polyenes) demonstrates an urgent need for alternative therapeutic strategies. The objective of this study was to evaluate the antifungal activity of novel organic derivatives from bioactive natural compound, including Naphthalene, Betulinic Acid (BA) [2]; Protocatechuic Acid (PCA) and Gallic Acid (GA), alone, or in combination with conventional antifungals to investigate synergistic effects that enhance their efficacy. Antifungal susceptibility assays were performed in *C. albicans* ATCC 10231 using the broth microdilution method, according to CLSI M27 guidelines, to determine Minimum Inhibitory Concentrations (MICs) and Minimum Fungicidal Concentrations (MFC). The interaction between BA derivatives and amphotericin B was assessed by checkerboard assay and fractional inhibitory concentration index (FICI). Naphthalene, PCA and GA derivatives showed low antifungal activity, falling within the moderate to weak bioactivity range (MIC ≥ 100 µg mL^{-1}; [3]). Among BA derivatives, BA-4 and BA-5 exhibited the best antifungal activity, with MIC values of 0,95 and 1,91 µg mL^{-1}, respectively, achieving 99% fungicidal activity at the same concentration. Furthermore, in combination assays, both compounds enhanced Amphotericin B activity, showing synergistic effects (FICI = 0.50), resulting in a decrease in MIC. Although most newly synthesized compounds exhibited limited antifungal potential, BA-4 and BA-5 demonstrated very strong bioactivity and fungicidal effects against *C. albicans*. The observed synergy with amphotericin B highlights their potential role as antifungal adjuvants and supports the further exploration of betulinic acid derivatives as viable approaches to overcoming antifungal resistance.

KEYWORDS: *candida albicans; antifungal therapy; bioactive natural compounds*

REFERENCES:
1Casalini et al., Lancet Microbe, 2024
2A.I. Nunes, Master thesis, ESS-IPP 2025
3Alves et al., BioMed Research International, 2021

Optimization of SPE-HPLC-FLD methodologies for the extraction and detection of steroid hormones in plasma

ISABELLA BRACCHI[1,2], PAULA PAÍGA[3], CRISTINA DELERUE-MATOS[3], DIOGO PESTANA[4], RITA NEGRÃO[1], ELISA KEATING[1] & VIRGÍNIA FERNANDES[3,5]

1. RISE-HEALTH, DEPARTMENT BIOMEDICINE, UNIT OF BIOCHEMISTRY, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, 4200-319 PORTO, PORTUGAL; 2. ESS, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 3. REQUIMTE/LAQV, INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL; 4. CHRC | NUTRITION AND METABOLISM, NOVA MEDICAL SCHOOL | FCM, NOVA UNIVERSITY LISBON, LISBON, PORTUGAL 5. RISE-HEALTH, CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA (TBIO), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL.

The quantification of steroid hormones, such as estradiol and ethinylestradiol, in plasma requires sensitive and reliable analytical methodologies[1]. This study presents the optimization of a bioanalytical method based on Solid-Phase Extraction (SPE) coupled with High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD). For sample extraction, Strata-X (polymeric reversed-phase) cartridges were used, where critical SPE parameters, including cartridge conditioning, matrix washing, and analyte elution, were evaluated. Chromatographic separation was achieved on a C18 stationary phase using a mobile phase of acetonitrile and 0.1% formic acid in ultrapure water. Both isocratic and gradient elution programs were tested to maximize resolution and peak shape. The method was validated for linearity, recovery, and precision (intra- and inter-day), meeting all established criteria for bioanalytical applications. This combined optimization resulted in improved selectivity and sensitivity for the determination of estradiol and ethinylestradiol in plasma (Figure 1), demonstrating a robust bioanalytical tool for monitoring these compounds in complex biological matrices.

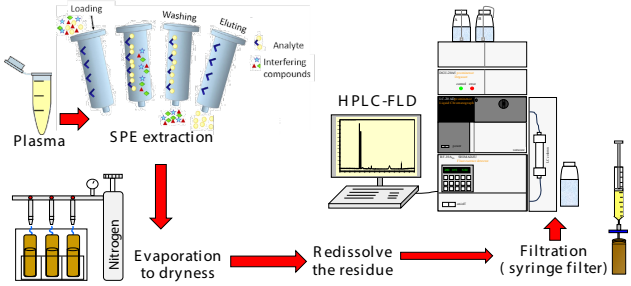

Figure 1. Overview of the SPE-HPLC-FLD methodology for steroid hormones analysis in plasma.

KEYWORDS: *high-performance liquid chromatography*; *steroid hormones*, *solid-phase extraction*

ACKNOWLEDGEMENTS:
This work received financial support from the Northern Regional Operational Program under the HEALTH-UNORTE project (NORTE-01-0145-FEDER-000039) and the FCT Fundação para a Ciência e a Tecnologia and by FCT/MCTES (Foundation for Science and Technology and Ministry of Science, Technology and Higher Education) (2023.03003.BD). Authors also thank the portuguese national funds (FCT/MECI, Fundação para a Ciência e a Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (DOI: 10.54499/UID/50006/2025).

REFERENCES:
1 Almeida, C; Nogueira J. M. F. J. Pharm. Biomed. Anal. 2006, 41, 1303-1311.

Unveiling ocean secrets: the antimicrobial power of azorean marine-derived actinomycetes

CATARINA M. LUÍS[1,2], TIAGO CORREIA[1,2], DANIELA PARREIRA[1,2], SARA MARGARIDA[1,2], RITA G. SOBRAL[1,2] & SUSANA P. GAUDÊNCIO[1,2]

1. ASSOCIATE LABORATORY I4HB, INSTITUTE FOR HEALTH AND BIOECONOMY, NOVA SCHOOL OF SCIENCE AND TECHNOLOGY, NOVA UNIVERSITY OF LISBON, CAMPUS CAPARICA, 2819-516 CAPARICA, PORTUGAL; 2. UCIBIO—APPLIED MOLECULAR BIOSCIENCES UNIT, CHEMISTRY AND LIFE SCIENCES DEPARTMENTS, NOVA SCHOOL OF SCIENCE AND TECHNOLOGY, NOVA UNIVERSITY OF LISBON, CAMPUS CAPARICA, 2819-516 CAPARICA, PORTUGAL

Actinomycetes thrive across a wide range of environments, from terrestrial soils and marine habitats to more extreme or underexplored niches such as caves, deserts, glaciers, and mangroves[1]. They are particularly renowned for their remarkable capacity to produce bioactive compounds, accounting for more than 70% of marketed antibiotics. Notable examples include streptomycin, tetracycline, chloramphenicol, erythromycin-like macrolides, and many other clinically relevant drugs[2].This study investigated the phylogenetic diversity of actinomycetes isolated from oceanic sediments collected off the Azores archipelago, including São Miguel and Santa Maria Islands and Formigas Islets, and evaluated their antimicrobial potential against two pathogenic bacteria: methicillin-resistant *Staphylococcus aureus* (MRSA) COL and *Escherichia coli* ATCC 35218. Using 16S rRNA sequencing, actinomycete isolates obtained from diverse sediment samples were taxonomically identified. A total of 224 strains across 15 genera, dominated by *Streptomyces*, were obtained from 558 samples collected via scuba and reel/grab methods (10–810 m depth). Antimicrobial screening revealed that 38 strains inhibited MRSA, while 2 showed activity against *E. coli*. These findings highlight Azorean marine environments as a rich and still underexplored reservoir of actinomycete diversity with significant antimicrobial potential. The predominance of activity against MRSA underscores the relevance of these isolates in the search for new agents targeting antibiotic-resistant pathogens. Overall, this study reinforces the value of investigating unique marine ecosystems for the potential discovery of novel bioactive compounds.

KEYWORDS: *actinomycetes*, *blue biotechnology*; *antimicrobial*; *one health*; *sustainability*

ACKNOWLEDGMENTS:
This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. FCT Project PTDC/QUI-QUI/101813/2008.

REFERENCES:
1 Sayed, A. M., Hassan, M. H. A., Alhadrami, H. A., Hassan, H. M., Goodfellow, M., & Rateb, M. E. (2020). *Journal of Applied Microbiology*, *128*(3), 630–657. https://doi.org/10.1111/jam.14386
2 De Simeis, D., & Serra, S. (2021). *Antibiotics*, *10*(5), 483. https://doi.org/10.3390/antibiotics10050483

Recovery and stabilization of recombinant Nucleic-acid biopharmaceuticals with ionic liquids

JOÃO L. FERNANDES, MIGUEL F. GALRINHO, ANA C. S. MARQUES, JOANA L. COSTA, ANA P. M. TAVARES, AUGUSTO Q. PEDRO, & MARA G. FREIRE

CICECO – AVEIRO INSTITUTE OF MATERIALS, CHEMISTRY DEPARTMENT, UNIVERSITY OF AVEIRO, AVEIRO, PORTUGAL;

Due to fast development times and efficient immune responses, messenger RNA (mRNA) vaccines were vital to combat the COVID-19 pandemic. Despite this, the widespread use of mRNA therapeutics is still challenged by high production costs and low stability of the finished product. The current gold standard for mRNA production is in vitro transcription (IVT) followed by several purification steps, ending in strict cold-chain storage at very low temperatures. This process is complex, expensive and dependent on reagents with limited availability[1]. Building on these limitations, this work focuses on developing new, integrated, and cost-effective downstream processes for bioproduced mRNA, particularly for mRNA extraction from yeast intracellular compartments. To achieve this, a set of bio-based ionic liquids (IL) was synthesized and characterized, and mRNA produced in a modified yeast strain according to standard protocols. Initial results revealed that ILs comprising carboxylic acids as anions were effective at yeast cell disruption and the consequent release of the target biopharmaceutical. Furthermore, through electrophoresis and RT-qPCR analysis it was possible to conclude that IL-mediated lysis yielded a similar amount of target mRNA to the control method used (Glass beads), but with superior scalability. It was additionally shown that aqueous solutions of the selected ILs are able to maintain the stability of nucleic acids, acting thus as preservation media of these biopharmaceuticals at room temperature. Overall, this work shows the beneficial role of ILs in developing integrated extraction-preservation methods for RNA.

KEYWORDS: *mrna vaccine; ionic liquid; bioproduction*

ACKNOWLEDGMENTS:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). This work was additionally funded by the EIC-Pathfinder YSCRIPT project with reference 101047214, supported by the budgets of the Horizon Europe Program. João L. Fernandes, Ana C. S. Marques, Miguel F. Galrinho and Joana L. Costa acknowledge FCT, respectively, for the PhD grants 2024.05338.BDANA, 2023.04854.BD, 2024.18530.PRT and 2025.04562.BDANA. Augusto Q. Pedro and Ana P. M. Tavares acknowledge FCT for the research contracts under the Scientific Stimulus – Individual Call, respectively, CEECIND/2020/02599 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) and CEECIND/2020/01867 (DOI 10.54499/2020.01867.CEECIND/CP1589/CT0013).

REFERENCES:
1Ali N, Rampazzo R de CP, Costa ADT, Krieger MA. Current Nucleic Acid Extraction Methods and Their Implications to Point-of-Care Diagnostics. Biomed Res Int. 2017;2017:1–13. doi:10.1155/2017/9306564.

Enhanced in vivo efficacy of Fluconazole against Candida glabrata via Lipossomal anti-PDR1 Antisense Therapy

PATRÍCIA SERRA[1,2,4], JOANA CASTRO[1], MARIANA HENRIQUES[2,3], DANIELA ARAÚJO[1], NUNO F. AZEVEDO[4], & SÓNIA SILVA[2,3]

1. INIAV, VILA DO CONDE, PORTUGAL; 2. CEB, BRAGA, PORTUGAL; 3. LABBELS, BRAGA/GUIMARÃES, PORTUGAL. 4. LEPABE, ALICE, PORTO, PORTUGAL.

Candida infections are a significant public health concern, with mortality and morbidity reaching 60%[1]. These infections not only prolong hospital stays but also impose a substantial financial burden on healthcare systems worldwide[2]. Among *Candida* species, *Candida glabrata*, recently reclassified as *Nakaseomyces glabratus*, poses a major clinical challenge due to its increasing resistance to conventional antifungal drugs, particularly azoles. Consequently, there is an urgent need for novel therapeutic strategies. Antisense therapy (AST) has emerged as a promising approach for the treatment of infectious diseases[3]. In this study, an antisense oligonucleotide (ASO) targeting *PDR1*, a transcription factor involved in the antifungal resistance of *C. glabrata*, was designed and synthesized with the aim of reversing fluconazole resistance. To enhance *in vivo* delivery, the ASO was encapsulated in lipid nanoparticles (LipoASOs)[4]. The safety and efficacy of these nanosystems were evaluated using the *Galleria mellonella* infection model, measuring larvae survival and health index[5]. *In vitro* assays confirmed that the anti-*PDR1* ASO reduced the expression of the target gene by approximately 30%, even under fluconazole-induced conditions. Moreover, the ASO enhanced growth inhibition of *C. glabrata* by at least 20% compared to the control, whether applied alone or in combination with fluconazole. While fluconazole alone did not improve *in vivo* outcomes, its combination with the LipoASOs significantly increased larval survival at 72 hours by around 30%. Overall, these findings show that the anti-*PDR1* ASO effectively suppresses *C. glabrata* growth and restores its susceptibility to azoles, especially when delivered via liposomal carriers. The outstanding *in vivo* performance of LipoASO–fluconazole combinations highlight their potential as a new strategy to overcome resistance and improve treatment in infections caused by multidrug-resistant fungal pathogens.

KEYWORDS: *antifungal resistance, rna-based therapeutics, liposomal delivery*

ACKNOWLEDGMENTS:
This study was supported by FCT under the scope of the strategic funding of UIDB/04469/2020, UIDB/04050/2020 units and Oligos4Resistance project 2023.16872.ICDT, by LABBELS LA/P/0029/2020; Associate Laboratory ARNET LA/P/0069/2020; LEPABE, UIDB/00511/2020 and UIDP/00511/2020; ALiCE LA/P/0045/2020). P.S. thanks FCT for the PhD Grant (2025.03604.BDANA). JC and DA, also thank FCT for the CEEC Individual (DOI: 10.54499/2022.06886.CEECIND/CP1737/ CT0001, DOI: 10.54499/2023.06040.CEECIND/CP2873/CT0001, respectively), and SS for the CEEC Institutional (https://doi.org/10.54499/CEECINST/00018/2021).

REFERENCES:
1Lamoth, F.; Lockhart, S.R.; Berkow, E.L.; Calandra, T. Changes in the Epidemiological Landscape of Invasive Candidiasis. Journal of Antimicrobial Chemotherapy 2018, 73, i4–i13.
2Wan Ismail, W.N.A.; Jasmi, N.; Khan, T.M.; Hong, Y.H.; Neoh, C.F. The Economic Burden of Candidemia and Invasive Candidiasis: A Systematic Review. Value in Health Regional Issues 2020, 21, 53–58.
3Araújo, D.; Azevedo, N.M.; Barbosa, A.; Almeida, C.; Rodrigues, M.E.; Henriques, M.; Silva, S. Application of 2′-OMethylRNA′ Antisense Oligomer to Control Candida Albicans EFG1 Virulence Determinant. Molecular Therapy - Nucleic Acids 2019, 18, 508–517.
4Araújo, D.; Gaspar, R.; Mil-Homens, D.; Henriques, M.; Silva, B.F.B.; Silva, S. Cationic Lipid-Based Formulations for Encapsulation and Delivery of Anti-EFG1 2′ OMethylRNA Oligomer. Medical Mycology 2022, 60.
5Araújo, D.; Mil-Homens, D.; Henriques, M.; Silva, S. Anti-EFG1 2′-OMethylRNA Oligomer Inhibits Candida Albicans Filamentation and Attenuates the Candidiasis in Galleria Mellonella. Molecular Therapy — Nucleic Acids 2022, 27, 517–523.

Chronic CGRP Administration Induces SexSpecific Hypersensitivity and Gut Immune Activation in Rats

LARA VIEIRA[1,2,3], MARIA FERREIRA[1,2,3], JOANA FERREIRA-GOMES[1,2,3], FANI L. NETO[1,2,3], ISABEL MARTINS[1,2,3]

1. DEPARTMENT OF BIOMEDICINE, UNIT OF EXPERIMENTAL BIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 2. IBMC, INSTITUTE OF MOLECULAR AND CELL BIOLOGY, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 3. I3S, INSTITUTE OF INVESTIGATION AND INNOVATION IN HEALTH, UNIVERSITY OF PORTO, PORTO, PORTUGAL.

Chronic migraine is a highly disabling neurological disorder characterized by recurrent, severe headaches and heightened sensory sensitivity. Calcitonin generelated peptide (CGRP)–driven activation of the trigeminovascular system remains the most widely studied mechanism, promoting vasodilation, neurogenic inflammation, and sensitization of trigeminal afferents. These processes contribute to peripheral and central sensitization, ultimately manifesting as cephalic and extracephalic hypersensitivity[1]. Increasing evidence implicates altered gut–brain communication in migraine pathophysiology, with CGRP emerging as a key mediator of this bidirectional axis[2]. We aimed to characterize a novel rat model of chronic migraine induced by two cycles of repeated CGRP administration, focusing on nociceptive sensitization, descending pain modulation, and intestinal alterations. Timecourse nociceptive responses were assessed using the Von Frey test. Descending pain modulation was evaluated through diffuse noxious inhibitory controls (DNIC), the rodent analogue of conditioned pain modulation. Colon and rectum histology was performed to quantify crypt depth, goblet cell density, and gutassociated lymphoid tissue. In females, CGRP induced cyclic peripheral sensitization, with periorbital allodynia at the end of each cycle and sustained hind paw allodynia. In males, CGRP produced only transient sensitization, limited to periorbital allodynia after the first cycle. DNIC responses revealed greater analgesia in CGRPtreated males than females, indicating impaired descending inhibition in females. Crypt depth did not differ between groups. CGRP increased goblet cell density in males compared with vehicle (14.0±2.1 vs 8.0±1.1). Lymphocytic aggregates were more frequent in controls but larger in CGRPtreated females. Preliminary immunophenotyping showed increased CD4+ lymphocyte infiltration in treated females relative to controls (44.9%±28.7 vs 29.4%±23.7). Repeated CGRP administration induces sexdependent hypersensitivity and altered pain modulation, alongside gut histological changes suggestive of immune activation. This model may provide a valuable tool for investigating gut–brain axis contributions to chronic migraine.

KEYWORDS: *migraine; cgrp; peripheral sensitization; central sensitization; gut.*

ACKNOWLEDGMENTS:
Grünenthal Pain Award, Fundação Grünenthal; Headache Research Grant *Dolore Capitis*, Sociedade Portuguesa de Cefaleias.

REFERENCES:
1 Goadsby, P. J. et al. Pathophysiology of Migraine: A Disorder of Sensory Processing. Physiol Rev 97, 553–622 (2017). https://doi.org/10.1152/physrev.00034.2015
2 Spekker, E. & Nagy-Grocz, G. All Roads Lead to the Gut: The Importance of the Microbiota and Diet in Migraine. Neurol Int 15, 1174–1190 (2023). https://doi.org/10.3390/neurolint15030073

Evaluation of the Concordance of Pharmacogenomic Information in the RCM and FI with the International Recommendations of CPIC and PharmGKB

NUNO ALMEIDA[1], ANA VASCONCELOS[1]; IVO LACERDA[1] & MARLENE SANTOS[2]

1. ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, PORTO, PORTUGAL; 2. REQUIMTE/LAQV, ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, PORTO, PORTUGAL

Introduction: Pharmacogenomics plays an increasing role in optimizing antiviral therapy, allowing the choice and dosage of drugs to be tailored to the patient's genetic profile. However, the integration of this information into regulatory documents, such as the Summary of Product Characteristics (SmPC) and the Patient Information Leaflet (PIL), remains inconsistent.

Objective: To evaluate the degree of concordance between the pharmacogenomic information presented in the SmPC and PIL of antiviral drugs with the international recommendations of the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the evidence levels reported in PharmGKB.

Methods: An analysis was conducted on a set of 146 marketed antiviral drugs, assessing the presence of pharmacogenomic recommendations in their respective SmPCs and PILs. These data were compared with CPIC guidelines and the pharmacogenomic evidence levels assigned by PharmGKB.

Results: Only 14 out of the 146 drugs analysed presented recommendations based on CPIC. Regarding PharmGKB evidence levels, most active substances exhibited level 3. Complete agreement with CPIC guidelines was observed in only 2 SmPCs, while other 5 did not consider any genetic polymorphisms. The remaining documents contained incomplete or partially aligned pharmacogenomic information relative to international recommendations.

Conclusion: The results show that, with the exception of abacavir, there is a discrepancy between the available pharmacogenomic evidence and its integration into the SmPCs of antiviral medicines. This misalignment reinforces the need for more comprehensive studies and effective harmonization between international guidelines and the content of SmPCs, promoting the adoption of personalized medicine.

KEYWORDS: *pharmacogenomics; antiviral; cpic; smpcs; abacavir; atazanavir; efavirenz*

Betulinic Acid Modulates Adipocyte Differentiation and Secretome Activity: Downstream Effects on Glioblastoma Cell Migration

AFONSO GOUVEIA[1,2], RICARDO FERRAZ[1,3], CRISTINA PRUDÊNCIO[1] & SÍLVIA FERNANDES[1,4]

1. CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA (TBIO)/REDE DE INVESTIGAÇÃO EM SAÚDE (RISE-HEALTH), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO (E2S|P.PORTO), PORTO, PORTUGAL; 2. MESTRADO EM METABOLISMO - BIOPATOLOGIA E EXPERIMENTAÇÃO, FMUP, PORTO, PORTUGAL; 3. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO, PORTUGAL; 4. ÁREA TÉCNICO- CIENTÍFICA DE ANATOMIA PATOLÓGICA, CITOLÓGICA E TANATOLÓGICA, ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO (E2S|P.PORTO), PORTO, PORTUGAL

Betulinic acid (BA) has been recognized for its potential to modulate tumor progression and adipocyte function, yet its effects on adipocyte secretome remodeling and the downstream consequences for glioblastoma behavior remain incompletely understood[1-4]. In this study, we investigated the influence of BA on adipocyte secretory activity and its functional impact on glioblastoma cell migration. Direct exposure of U251 glioblastoma cells to BA significantly reduced cell migration, confirming its intrinsic anti-migratory potential. To further explore microenvironmental interactions, adipocyte-conditioned media (ACM) derived from BA-treated adipocytes was evaluated for its effects on glioblastoma behavior. Secretome analysis demonstrated that BA altered matrix metalloproteinase (MMP) expression and reduced gelatinolytic activity, indicating significant modulation of extracellular matrix–related proteolytic pathways. Functional assessment using wound healing assays revealed that ACM from BA-treated adipocytes did not consistently suppress glioblastoma migration. Intermediate BA concentrations were associated with increased migratory behavior relative to basal conditions, although differences compared with control ACM were not statistically significant. Additionally, transwell migration assays did not demonstrate significant differences between treatment conditions, suggesting that BA-induced alterations in adipocyte secretome composition do not uniformly translate into measurable changes in glioblastoma migratory capacity across different experimental models. These findings indicate that modulation of gelatinolytic pathways alone is insufficient to predict glioblastoma cell migration and support the contribution of additional adipocyte-derived signalling mechanisms in regulating tumor behavior. Overall, BA induces substantial remodelling of adipocyte secretory function, producing complex and non-linear downstream effects on glioblastoma cells. This study highlights the importance of tumor microenvironment crosstalk and underscores the need to evaluate multiple regulatory pathways when considering the therapeutic potential of BA and related bioactive compounds.

KEYWORDS: *betulinic acid, obesity, glioblastoma multiforme*

REFERENCES:
1 Fernandes S, Vieira M, Prudêncio C, Ferraz R. International Journal of Molecular Sciences. 2024;25(4):2108.
2Lo W-L, Hsu T-I, Yang W-B, Kao T-J, Wu M-H, Huang Y-N, et al. Cancers. 2020;12(4):981.
3Coricovac D, Dehelean CA, Pinzaru I, Mioc A, Aburel OM, Macasoi I, et al. Int J Mol Sci. 2021;22(9).
4Liao L, Liu C, Xie X, Zhou J. Journal of food biochemistry. 2020;44(7):e13278.

Development and Characterization of Nanostructure Lipid Carriers Containing Fisetin

MARA MACEDO[1], ANA CAMILA MARQUES[2,3], ANA DANIELA ALVES[2,3], MARIA HELENA AMARAL[2,3] & JORGE FERNANDO BRANDÃO PEREIRA[4]

1. FACULTY OF SCIENCE AND TECHNOLOGY OF UNIVERSITY OF COIMBRA, COIMBRA, PORTUGAL; 2. UCIBIO – APPLIED MOLECULAR BIOSCIENCES UNIT, MEDTECH -LABORATORY OF PHARMACEUTICAL TECHNOLOGY, DEPARTMENT OF DRUG SCIENCES, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGA; 3. ASSOCIATE LABORATORY I4HB, INSTITUTE FOR HEALTH AND BIOECONOMY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL; 4. CERES, DEPARTMENT OF CHEMICAL ENGINEERING, FACULTY OF SCIENCE AND TECHNOLOGY OF UNIVERSITY OF COIMBRA, COIMBRA, PORTUGAL

Melanoma is the most dangerous and deadliest skin cancer, representing 5% of all skin cancers, with a patient's median survival time of 9 months. The incidence of cutaneous melanoma is increasing all over the world, gradually and worryingly. The main current treatment for melanoma is surgery, considered a risky incisive intervention. This disease remains unresolved, as even the most recent alternatives have limitations in terms of curative and palliative effects, in addition to serious side events.[1] Fisetin is a naturally occurring flavonoid, present in various fruits and vegetables. The anti-cancer potential of fisetin has been confirmed to target several cells signaling pathways including angiogenesis, apoptosis, inflammation, cell cycle and PI3K/AKT/mTOR signaling pathway. However, the usefulness of this compound is due to its low bioavailability and rapid metabolism.[2] Nanotechnology-based drug delivery systems are a promising strategy for overcoming these limitations.[3] The purpose of this work is to develop Nanostructured Lipid Carriers (NLC) capable of improving fisetin's therapeutic efficacy against Melanoma. NLCs were prepared by emulsifying the molten lipid phase containing fisetin with the aqueous phase containing a surfactant, followed by ultrasonication.[3] Then, the NLCs were characterized by Dynamic Light Scattering, Differential Scanning Calorimetry, Fourier-Transform Infrared Spectroscopy and High-Performance Liquid Chromatography. The mean size of empty NLCs and loaded with fisetin was, approximately, 190 nm. All formulations showed negative zeta potential values and good physical stability after 30 days of storage at 4°C and 25°C. The evaluation of the encapsulation efficiency showed that approximately 88% of fisetin was incorporated, likely in an amorphous or molecularly dispersed state, within the semi-crystalline lipid matrix.

KEYWORDS: *melanoma; fisetin; nanostructured lipid carriers*

ACKNOWLEDGMENTS:
This work was supported by national funding from FCT– Fundação para a Ciência e a Tecnologia, I.P., under projects UIDP/04378/2020, UIDB/04378/2020, UID/04378/2025, and UID/PRR/04378/2025 (UCIBIO), LA/P/0140/2020 (i4HB), and the project "CD-CORE: Cancer Diagnosis, Therapies and Lifestyle – Strategies to Overcome Chemotherapy Resistance and Long-Term Adverse Effects" (Operation No. NORTE2030-FEDER-02707400), co-funded by the European Regional Development Fund (ERDF) through the Northern Regional Programme 2021–2027 [NORTE2030].

REFERENCES:
1 Sias-Fonseca, L.; Costa, P. C.; Saraiva, L.; Alves, A.; Amaral, M. H. *Colloids Interfaces* 2025, 9, 53.
2 Rahmani, A. H.; Almatroudi, A.; Allemailem, K. S.; Khan, A. A.; Almatroodi, S. A. *Molecules* 2022, 27, 9009.
3 Marques, A. C.; da Costa, P. C.; Gonçalves, H.; Catita, J.; Velho, S.; Amaral, M. H. J. Drug Deliv. Sci. Technol. 2025, 114, 107571.

Bioactive Contact Lens Incorporating Umbilical Cord-Derived Mesenchymal Stem Cell Secretome for Corneal Regeneration

ANA COUTINHO[1,2], ANA CATARINA SOUSA[1,3,4,5,6,7] & ANA COLETTE MAURÍCIO[1,3,4,5]

1. DEPARTAMENTO DE CLÍNICAS VETERINÁRIAS, INSTITUTO DE CIÊNCIAS BIOMÉDICAS DE ABEL SALAZAR (ICBAS), UNIVERSIDADE DO PORTO (UP), PORTO, PORTUGAL; 2. ESCOLA SUPERIOR DE SAÚDE DO POLITÉCNICO DO PORTO (E2S), PORTUGAL; 3. CENTRO DE ESTUDOS DE CIÊNCIA ANIMAL (CECA), INSTITUTO DE CIÊNCIAS, TECNOLOGIAS E AGROAMBIENTE (ICETA) DA UP, PORTUGAL; 4. ASSOCIATE LABORATORY FOR ANIMAL AND VETERINARY SCIENCE (AL4ANIMALS), PORTUGAL; 5. LAQV REQUIMTE, INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR, UNIVERSIDADE DO PORTO, PORTO, PORTUGAL; 6. RISE-HEALTH, SCHOOL OF MEDICINE AND BIOMEDICAL SCIENCES, FERNANDO PESSOA UNIVERSITY, FERNANDO PESSOA TEACHING AND CULTURE FOUNDATION, GONDOMAR, PORTUGAL, 7. BIOMEDICAL AND HEALTH SCIENCES (FP-BHS), INSTITUTO DE INVESTIGAÇÃO, INOVAÇÃO E DESENVOLVIMENTO FERNANDO PESSOA (FP-I3ID), FUNDAÇÃO ENSINO E CULTURA FERNANDO PESSOA, PORTO, PORTUGAL.

Corneal injuries and degenerative corneal disorders remain a significant clinical challenge, as impaired tissue regeneration can lead to corneal opacity, neovascularization, and progressive vision loss [1,2]. Current therapeutic approaches, including topical eye drops and corneal transplantation, are limited by poor bioavailability, insufficient regenerative potential, and the risk of immune rejection [3]. Therefore, there is a pressing need for innovative, non-invasive strategies capable of restoring corneal function. In this context, conditioned medium derived from umbilical cord mesenchymal stem cells (UC-MSCs), rich in bioactive factors (secretome), has shown promising regenerative and immunomodulatory effects in corneal repair. This study aims to develop a bioactive contact lens as a non-invasive therapeutic platform for corneal regeneration. The system is based on a hydrogel composed of polyvinyl alcohol (PVA), hyaluronic acid (HA), and UC-MSC-derived conditioned medium. Prior to incorporation, the secretome will be characterized to identify key biomarkers associated with immunomodulation and tissue regeneration and validated in vitro through cytocompatibility and cell migration (scratch) assays. The bioactive contact lenses will be fabricated using mold-based techniques to ensure reproducible geometry and structural consistency. Within the hydrogel matrix, PVA provides mechanical stability and flexibility, while HA enhances hydration, biocompatibility, and mimics native corneal properties, contributing to comfort and biological performance. This platform enables sustained and localized delivery of bioactive factors directly to the injured corneal surface. The resulting hydrogel will undergo physicochemical characterization using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Biological performance will be evaluated in vitro using corneal epithelial cells under conditions that mimic the ocular environment. Overall, this work proposes a promising cell-free regenerative strategy for corneal wound healing, with the potential to enhance epithelial repair, reduce fibrosis, and improve visual outcomes.

KEYWORDS: *contact lens, corneal repair, secretome, umbilical cord mesenchymal stem/stromal cells*

ACKNOWLEDGMENTS:
This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

REFERENCES:
1B. Barrientez, S. E. Nicholas, A. Whelchel, R. Sharif, J. Hjortdal, and D. Karamichos, "Corneal Injury: Clinical and Molecular Aspects," Exp. Eye Res., vol. 186, p. 107709, Sep. 2019, doi: 10.1016/J.EXER.2019.107709.
2S. Kacham et al., "Human Umbilical Cord-Derived Mesenchymal Stem Cells Promote Corneal Epithelial Repair In Vitro," Cells, vol. 10, no. 5, May 2021, doi: 10.3390/CELLS10051254
3J. Kwon et al., "Emerging Treatments for Persistent Corneal Epithelial Defects," Vision 2025, Vol. 9, vol. 9, no. 2, Mar. 2025, doi: 10.3390/VISION9020026.

Sensing the mind: label-free miRNA-based sensor as a diagnostic tool for neurodevelopmental disorders

EDUARDA PEREIRA[1,2,3,4,5,6], RENATA SANTOS[1], CATARINA PRIOR[7], MARLENE SANTOS[2,3,4,5], ANA LUÍSA TEIXEIRA[2,3,4] & M. FÁTIMA BARROSO[1]

1. REQUIMTE|LAQV, INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL; 2. MOLECULAR ONCOLOGY AND VIRAL PATHOLOGY GROUP, IPO PORTO RESEARCH CENTER (CI-IPO PORTO), PORTUGUESE ONCOLOGY INSTITUTE OF PORTO (IPO PORTO); 3. RISE - ASSOCIATE LABORATORY (HEALTH RESEARCH NETWORK); 4. PORTO COMPREHENSIVE CANCER CENTRE RAQUEL SERUCA (PORTO.CCC), PORTUGAL; 5. REQUIMTE|LAQV, ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL; 6. FACULTY OF SCIENCES OF THE UNIVERSITY OF PORTO, PORTO, PORTUGAL. 7. CENTRO HOSPITALAR UNIVERSITÁRIO DE SANTO ANTÓNIO, UNIDADE LOCAL DE SAÚDE DE SANTO ANTÓNIO, UNIDADE DE NEURODESENVOLVIMENTO.

Mental health disorders, including Attention-Deficit/Hyperactivity Disorder (ADHD) and autism spectrum disorder (ASD), are global public health concerns that lack accurate and fast diagnostic tools. Recent evidence highlights miR-151a-3p as a promising circulating biomarker, as its levels in plasma and saliva are associated with neurodevelopmental disorders. However, current detection methods like RT-qPCR are expensive and time-consuming, which creates a demand for innovative *point-of-care* solutions. Highly sensitive electrochemical biosensor for the direct detection of miR-151a-3p emerge as promising alternatives, in gold screen-printed electrodes functionalized with a specific DNA-151a-3p capture probe. The biosensor's assembly and the hybridization events were monitored using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Preliminary results indicate that the biosensor exhibits high selectivity and sensitivity, distinguishing the target miR-151a-3p from non-complementary sequences within biologically relevant concentration ranges. This label-free approach offers a significant advantage over traditional assays by providing a cost-effective, fast, and portable diagnostic tool.

KEYWORDS: *mir-151a-3p; electrochemical biosensor; neurodevelopmental disorders*

ACKNOWLEDGEMENTS:
This work received financial support from the PT national funds (FCT/MECI) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and from the project "Challenging the Analytical Diagnosis of Neurodevelopmental Disorders: Pioneering Neurochemical Devices", funded by FEDER funds and PT funds by FCT/MECI, operation no. 15875, with reference COMPETE2030-FED-ER-00699000. Eduarda Pereira (2024.01697.BDANA) is grateful to FCT and the European Union (EU) for their grants financed by POPH-QREN-Tipologia 4.1-Formação Avançada, funded by Fundo Social Europeu (FSE) and Ministério da Ciência, Tecnologia e Ensino Superior (MCTES). M. Fátima Barroso thanks the TENURE – FCT Tenure Program – 1st Edition (Reference 2023.15797.TENURE.001). with financial support from FCT/MCTES through national funds.

A Proof-of-Concept Aptamer Platform Combining In Silico Design, Optical Validation, and Electrochemical Detection of the Fish Allergen β-Parvalbumin

ANA G. ABRIL[1], ADRIÁN SÁNCHEZ-VISEDO[2], MANUEL PAZOS[3], FLEMMING MORSCH[4], GEORGIOS POULOS[5], CHRISTIAN CODE[5], MARTA PRADO[6], MÓNICA CARRERA[3] & RUI CAMPOS[2]

1. DEPARTMENT OF FUNCTIONAL BIOLOGY AND HEALTH SCIENCES, AREA OF MICROBIOLOGY, UNIVERSIDADE DE VIGO, VIGO, SPAIN; 2. INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY (INL), BRAGA, PORTUGAL; 3. DEPARTMENT OF FOOD TECHNOLOGY, SPANISH NATIONAL RESEARCH COUNCIL (CSIC), INSTITUTE OF MARINE RESEARCH (IIM-CSIC), VIGO, SPAIN; 4. TECHNICAL UNIVERSITY OF DENMARK, KONGENS LYNGBY, DENMARK; 5. SCIENTEK APS, FRUEBJERGVEJ, COPENHAGEN, DENMARK; 6. FOOD HYGIENE, INSPECTION AND CONTROL LABORATORY (LHICA), DEPARTMENT OF ANALYTICAL CHEMISTRY, NUTRITION AND BROMATOLOGY, VETERINARY SCHOOL, UNIVERSITY OF SANTIAGO DE COMPOSTELA, LUGO, SPAIN

Ensuring food safety is a critical concern in the modern food industry, as the presence of allergens, toxins, and pathogens can pose significant risks to consumer health. Beta-parvalbumin (β-PRVB) is a calcium-binding protein and the main fish allergen present in raw and processed food products, triggering severe allergic reactions in sensitive individuals. In this research work, we explore the development of an electrochemical aptamer-based biosensor for the detection of β-PRVB[1]. The biosensor development began with the *in silico* design of aptamers targeting β-PRVB, which were assessed for their docking and binding capabilities. This was followed by validation using a fluorescence quenching assay, ultimately leading to the creation of an electrochemical aptamer-based sensor utilizing the selected aptamer. The sensor showed a very low limit of detection (LOD) of 3.3 µg/mL and a limit of quantification (LOQ) of 10 µg/mL, with its specificity being tested with beta-lactoglobulin (β-Lact). Samples of commercial processed seafood products were analyzed to determine the effectiveness of the sensor, and the results were validated.

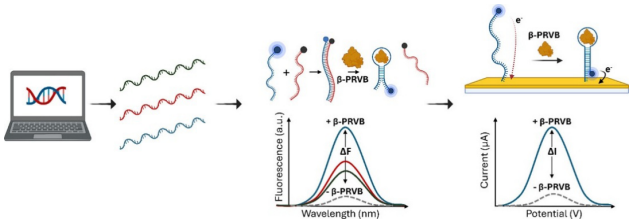

Figure1. *In Silico* Design, Optical Validation, and Electrochemical Detection of the Fish Allergen β-Parvalbumin

KEYWORDS: *beta-parvalbumin (β-prvb), electrochemical aptamer-based biosensor (e-ab), food safety.*

REFERENCES:
1Abril, A. G.; Sánchez-Visedo; A., Pazos, M.; Morsch, F.; Poulos, G.; Code, C.; Prado, M.; Carrera, M. & Campos, R. ACS Sensors. 2026, 11(3), 2130-2141.

Development, and characterization of an oral Budesonide-loaded formulation for the treatment of oral mucositis

CHIARA AMATA[1,2,3,#], CHIARA GIUSTO[1,2,3,#], FRANCISCA RODRIGUES[1,2], PAULO COSTA[1,2]

1. UCIBIO – APPLIED MOLECULAR BIOSCIENCES UNIT, MEDTECH-LABORATORY OF PHARMACEUTICAL TECHNOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL UNIVERSIDADE DO PORTO, FACULTY OF PHARMACY, PORTO, PORTUGAL. 2. I4HB – ASSOCIATE LABORATORY INSTITUTE FOR HEALTH AND BIOECONOMY, FACULTY OF PHARMACY, UNIVERSITY OF PORTO, PORTO, PORTUGAL 3. UNIVERSITY OF MESSINA, CHEMISTRY AND PHARMACEUTICAL TECHNOLOGY, CHIBIOFARAM.

# - THE AUTHORS CONTRIBUTED EQUALLY TO THE PRESENT WORK.

Oral mucositis is a common and dose-limiting complication of chemotherapy and radiotherapy, characterized by inflammation and ulceration of the oral mucosa [1]. Due to its localized nature, it represents an ideal target for site-specific drug delivery strategies aimed at improving local drug availability and prolonging residence time within the oral cavity [2]. In this study, budesonide-loaded multiparticulate pellets were developed using the extrusion–spheronization technique. The formulation was based on a physical mixture of mannitol and microcrystalline cellulose (MCC, Avicel® PH 102), where MCC was selected as the main structural excipient due to its high water-retention capacity and ability to provide adequate plasticity to the wet mass, while mannitol was used to improve powder flowability [3,4]. Powder blending was performed using a Caleva MULTI LAB CLASSIC system. The wet mass was obtained by controlled addition of an aqueous PVP solution as binder, followed by extrusion and spheronization using a Caleva system, evaluating preliminary different spheronization times to optimize pellet morphology [5]. Coating was performed using a hydroxypropyl methylcellulose (HPMC) and polyethylene glycol 400 (PEG 400) dispersion in a Caleva MINI COATER, with the aim of modulating surface properties and drug release behavior [6]. Powder flowability was assessed using a powder flow tester for mannitol, MCC, and their mixture in order to characterize the flow behavior of the solid starting system. Viscosity and rheological properties of PVP and HPMC solutions were evaluated using a Kinexus rheometer. Thermal properties of mannitol, MCC, and budesonide were investigated by DSC. The obtained granules were further characterized in terms of hardness, friability, particle size, and morphological analysis. The developed pellets showed good mechanical strength, adequate morphological uniformity, and suitable processability. These results support the extrusion–spheronization as a promising technique for the development of budesonide-loaded pellets for the local treatment of oral mucositis.

KEYWORDS: *budesonide, oral mucositis, oral formulations, wet granulation, extrusion–spheronization.*

FUNDING:
Francisca Rodrigues was supported by FCT - Fundação para a Ciência e a Tecnologia, I.P under the TENURE program (2023.14718.TENURE.016). The authors acknowledge the support from the Aliança para a Investigação e Ensino Superior, and the funding received by FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 (DOI:10.54499/UIDP/04378/2020) and UIDB/04378/2020 (DOI:10.54499/UIDB/04378/2020) of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI:10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

REFERENCES:
1Bell, A.; Kasi, A. Oral Mucositis. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2026.
2Dionísio, T.; Brandão, P.; Machado, V.; Mendes, J.J.; Fonte, P.; Botelho, J. In *Drug Delivery Systems for Wound Healing*; Elsevier, 2024; pp. 173–196 ISBN 978-0-323-90081-2.
3Sakr, W.F.; Ibrahim, M.A.; Alanazi, F.K.; Sakr, A.*Saudi Pharm J* **2012**, *20*, 9–19, doi:10.1016/j.jsps.2011.04.007.
4Goyanes, A.; Souto, C.; Martínez-Pacheco, R. . *Pharm Dev Technol* **2010**, *15*, 626–635, doi:10.3109/10837450903419653.
5Sinha, V.R.; Agrawal, M.K.; Agarwal, A.; Singh, G.; Ghai, D. *CRT* **2009**, *26*, doi:10.1615/CritRevTherDrugCarrierSyst.v26.i3.20.
6Ghadermazi, R.; Hamdipour, S.; Sadeghi, K.; Ghadermazi, R.; Khosrowshahi Asl, *Food Sci Nutr* **2019**, *7*, 3363–3377, doi:10.1002/fsn3.1206.

Evaluation of the functional properties of kombucha-derived Brettanomyces yeasts

LARA AREAL-HERMIDA[1], PEDRO COELHO [2,3], ÁNGELES PICHARDO-GALLARDO[1], CRISTINA PRUDÊNCIO [2,3] & CARMEN SIEIRO[1]

1. DEPARTAMENTO DE BIOLOXÍA FUNCIONAL E CIENCIAS DA SAÚDE. ÁREA DE MICROBIOLOXÍA. UNIVERSIDADE DE VIGO. LAGOAS-MARCOSENDE 36310 VIGO. SPAIN; 2. CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA (TBIO)/REDE DE INVESTIGAÇÃO EM SAÚDE (RISE-HEALTH), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL; 3. ATC CIÊNCIAS QUÍMICAS E DAS BIOMOLÉCULAS, ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL

Kombucha is a fermented beverage traditionally produced from tea and widely associated with beneficial health effects. These properties have been mainly attributed to the fermentable substrate and, to a lesser extent, to the microorganisms involved in the fermentation process. Therefore, the characterization of kombucha-associated microorganisms is essential to better understand and validate their potential health benefits. In this study, four probiotic yeast strains isolated from kombucha tea were identified, by the PCR-RFLP analysis of the ribosomal ITS region and the sequence of the D1/D2 domain of the 26S rDNA, as *Brettanomyces bruxellensis* (UVI55 and UVI56) and *B. anomalus* (UVI57 and UVI58). The evaluated strains exhibited remarkable antioxidant capacity, the ability to hydrolyze bile salts, and antimicrobial activity against several key pathogens. Additionally, they displayed significant cytotoxic effects across various tumor cell lines. Notably, in the *Caenorhabditis elegans* in vivo model, strain UVI56 significantly extended the nematode's lifespan and conferred protection against *S. enterica* infection. Although additional in vivo studies are necessary to further elucidate the underlying biological mechanisms and validate the therapeutic potential of these yeasts, the present findings highlight the relevance of *Brettanomyces* species as key members of the kombucha microbial consortium. These strains exhibit multiple biological activities of potential biomedical interest and may contribute synergistically to the health-promoting properties traditionally associated with kombucha beverages.

KEYWORDS: *kombucha, yeasts, brettanomyces, antimicrobial activity, cytotoxicity, longevity.*

FUNDING:
This study was supported by Agencia Estatal de Investigación from the Spanish Ministerio de Ciencia e Innovación (grant number PID2022-138330OB-100), Xunta de Galicia (grant number GPC-ED431B2023/35), and Universidade de Vigo (grant number 22VI-08). LA-H was the recipient of a Xunta de Galicia predoctoral fellowship (ED481A-2020/191).

REFERENCES:
1 Areal-Hermida, L., Coelho, P., Pichardo-Gallardo, A., Prudêncio, C. & Sieiro, C. (2024). Potential probiotic and functional properties of Brettanomyces strains Isolated from kombucha tea. Front. Microbial, 15. DOI=10.3389/fmicb.2024.1415616

Toward greener materials with enzyme-assisted polydopamine coatings

INÊS S.R. VIEIRA, JOÃO C.F. NUNES, MARA G. FREIRE, MÁRCIA C. NEVES & ANA P.M. TAVARES

CICECO – AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, 3810-193 AVEIRO, PORTUGAL

Polydopamine (PDA) has emerged as a versatile bioinspired material due to its strong adhesion, biocompatibility, antioxidant properties and broad applicability in surface functionalization, especially in biomedical contexts(1,2). In this sense, laccase-mediated dopamine polymerization has been proposed as a more sustainable and controlled alternative to conventional alkaline self-polymerization, which is typically slower, relies on harsher conditions and may be unsuitable for sensitive substrates (1,2). Studies have shown that laccase can catalyze PDA formation under mild and aqueous conditions, allowing coating deposition at acidic pH and improving process control (1,2). In parallel, the recombinant production of laccase in *Komagataella phaffii* demonstrated that the enzyme can be efficiently produced, purified and applied in PDA coating processes, supporting the scalability and industrial relevance of this approach(3). Based on these advances, this work aims to develop sustainable PDA-coated materials, to obtain functional surfaces with tunable wettability, adhesion and bioactivity (2,3). These materials may be explored for biomedical applications such as wound dressings, antimicrobial surfaces, wearable biosensors and other medical devices(1). The materials were characterized through FTIR, SEM, contact angle analysis and surface evaluation, in order to relate coating conditions with the final material performance. This work combines green chemistry, enzyme catalysis and biomaterials engineering to support the development of innovative and environmentally friendly platforms for biomedical use.

KEYWORDS: *polydopamine, laccase, dopamine polymerization, coating*

ACKNOWLEDGEMENTS:
This work was financially supported by Fundação para a Ciência e Tecnologia, Exploratory Project 2024.16757.PEX. This work was funded by FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2030 in the framework of the project COMPETE2030-FEDER-00771100 (Refª FCT: 16538).This work was developed within the scope of the project CICECO Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC).

REFERENCES:
1Magalhães FF, Pereira AF, Freire MG, Tavares APM. New liquid supports in the development of integrated platforms for the reuse of oxidative enzymes and polydopamine production. Front Bioeng Biotechnol. 2022 Nov 28;10. doi:10.3389/fbioe.2022.1037322
2Magalhães FF, Alfieri ML, Romanyuk K, Kholkin A, Freire MG, Napolitano A, et al. Laccase-mediated dopamine polymerization: a bioinspired and sustainable coating technology. Prog Org Coat. 2025 Oct 1;207. doi:10.1016/j.porgcoat.2025.109399
3Magalhães FF, Neves MC, Pedro AQ, Freire MG, Santos-Ebinuma VC, Tavares APM. Recombinant laccase biosynthesis for efficient polydopamine coating. Biochem Eng J. 2024 Dec 1;212. doi:10.1016/j.bej.2024.109483

Sustainable development: an educational laboratory exercise using bacteriophages

DULCE TEIXEIRA, MÓNICA VIEIRA & CRISTINA PRUDÊNCIO

CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY RESEARCH (TBIO)/HEALTH RESEARCH NETWORK (RISE-HEALTH), ESS, POLYTECHNIC OF PORTO, DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200-072, PORTO, PORTUGAL.

Sustainable development is a model that seeks to balance growth, social inclusion, and environmental protection, ensuring present and future well-being. The United Nations 17 Sustainable Development Goals (SDGs) guide this goal until 2030. Sustainable biotechnology is a branch that refers to the set of techniques and processes that use living organisms, their parts, or derivatives to develop products and solutions that provide economic, social, and environmental benefits. The growing development of the techniques that use living organisms, alongside the multidisciplinary nature of a career in medicinal biotechnology, underscores the need for students in this sector to acquire a robust understanding of sustainable biotechnology as a fundamental component of their education. To this end, we propose a class focused on bacteriophages and their added value, covering five laboratory sessions: (i) design of a strategy for the recovery, enrichment, and isolation of a bacteriophage; (ii) extraction and recovery of bacteriophages from an environmental water sample; (iii) bacteriophage enrichment (*Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 1457, *Escherichia coli* ATCC 25922, and *E. coli* ATCC 8739); (iv) isolation and propagation of bacteriophages; and (v) research on the applicability of bacteriophages in the field of sustainable development. The major goals of the present work were (i) to provide students with the necessary tools to adapt protocols in the context of a laboratory class; (ii) to assess the students' skills to carry out the assays in accordance with the expected results, and (iii) to foster awareness for sustainable development. All assays were performed by students of the B.Sc in Medicinal Biotechnology. The results showed that *Escherichia coli* ATCC 8739 was the only strain that allowed the isolation of bacteriophages. Overall, these laboratory sessions demonstrated the practical implementation of sustainability concepts while also expanding the knowledge of biotechnological laboratory techniques.

KEYWORDS: *laboratory exercise*, *sustainable biotechnology*, *sustainable development goals (sdgs)*, *bacteriophages*

REFERENCES:

1Imara, K., & Altinay, F. (2021). Integrating education for sustainable development competencies in teacher education. *Sustainability*, *13*(22), 12555. https://doi.org/10.3390/su132212555

2Abdullahi, A. M., Hussein, H. A., Ahmed, M. Y., Hussein, O. A., & Warsame, A. A. (2024). The impact of education for sustainable development on university students' sustainability behavior: A case study from undergraduate students in Somalia. *Frontiers in Education*, *9*, 1413687. https://doi.org/10.3389/feduc.2024.1413687

3Runa, V., Wenk, J., Bengtsson, S., Jones, B. V., & Lanham, A. B. (2021). Bacteriophages in biological wastewater treatment systems: Occurrence, characterization, and function. *Frontiers in Microbiology*, *12*, 730071. https://doi.org/10.3389/fmicb.2021.730071

Sustainable extraction of bioactive actinidin protease from kiwi waste for biotechnological applications

CAROLINA M. SAÚDE[1], ANA P. M. TAVARES[1]*, AUGUSTO Q. PEDRO[1], MARA G. FREIRE[1], FILIPE S. BUARQUE[1,2]

1. CICECO – AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, 3810-193 AVEIRO, PORTUGAL; 2. BIOCHEMICAL ENGINEERING DEPARTMENT, SCHOOL OF CHEMISTRY, FEDERAL UNIVERSITY OF RIO DE JANEIRO, BRAZIL.

Kiwifruit's industrial processing results in large quantities of waste containing added-value proteins.[1] Particularly, the cysteine protease actinidin can be found in kiwi peel residues and has been noted for its bioactive potential.[2] This enzyme aids protein digestion and leads to faster amino acid absorption,[2] promoting satiation after eating. For this reason, it can be incorporated into digestive supplements and functional health formulations.[3] Accordingly, this work aimed to extract the actinidin protease from kiwi peel waste using aqueous solutions of biocompatible cholinium-based ionic liquids (ILs). When adequately designed, ILs are promising solvents to enhance the extraction, preserve proteins, and improve their stability and biological activity.[4] For this purpose, the extraction parameters were optimized by evaluating the effect of the IL anion type, and by different times, agitations, temperatures, IL concentrations, and solid-liquid ratios. The best IL exhibited the highest protease activity at 1.245 U L^{-1}. All the ILs showed higher proteolytic activities than the phosphate buffer pH 7 (0.405 U L^{-1}), used as the traditional method. Furthermore, it was found that the actinidin extraction is enhanced at the optimised conditions, showing about 13-fold more activity than the traditional extraction method, which demonstrates the potential of cholinium-based ILs to keep or even superactivate the enzyme's activity.

KEYWORDS: *kiwifruit peel, actinidin, cholinium-based ionic liquids, proteolytic activity*

ACKNOWLEDGEMENTS:
This work was developed within the scope of the project CICECO Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC).

REFERENCES:
1F. Chamorro, M. Carpena, M. Fraga-Corral, M. Prieto, J. Simal-Gandara, Food Chem. 2022, 370, 131315.
2D.P. Richardson, J. Ansell, L.N. Drummond, Eur J Nutri. 2018, 57, 2659–2676.
3V. Dihman, V. Chauhan, S. Kanwar, D. Singh, H. Pandey, Bulletin of the National Research Centre. 2021, 45.
4R. Patel, M. Kumari, A.B. Khan. Appl Biochem Biotechnol. 2014, 172, 3701-3720.

Insights into the structure and activity of LONP2, a peroxisomal AAA+ ATPase

ANA RITA SILVA[1,2,3], TÂNIA FRANCISCO[1,2], TONY RODRIGUES [1,4,5], ANA G. PEDROSA[1,2], MARC FRANSEN[3] & JORGE E. AZEVEDO [1,2]

1. I3S/IBMC – INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE/ INSTITUTO DE BIOLOGIA MOLECULAR E CELULAR – UNIVERSIDADE DO PORTO 2. INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR (ICBAS), UNIVERSIDADE DO PORTO 3. DEPT. CELLULAR AND MOLECULAR MEDICINE, KATHOLIEKE UNIVERSITEIT LEUVEN 4. RISE-HEALTH, CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA(TBIO), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO 5. CIÊNCIAS QUÍMICAS E DAS BIOMOLÉCULAS, ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO

Peroxisomes are single membrane-bound organelles that participate in a wide range of metabolic pathways, often involving multiple H_2O_2-producing oxidases. The large amounts of H_2O_2 generated inside these organelles, combined with the absence of protein repair systems renders peroxisomal proteins particularly susceptible to oxidative damage. Notably, distinct peroxisomal matrix proteins were shown to display heterogeneous half-lives, pointing to the existence of Protein Quality Control mechanisms (PQC) that degrade damaged proteins. In mammalian peroxisomes, two proteases were identified: TYSND1 (Peroxisomal leader peptide processing protease) and LONP2 (Lon protease homolog 2). TYSND1 cleaves flexible loops within some peroxisomal proteins and the type 2 peroxisomal targeting signals; however its restricted substrate specificity makes a role in PQC system unlikely. In contrast, LONP2 belongs to the AAA+ family of ATP- dependent proteases, a family typically involved in PQC. Structurally, LONP2 comprises an N-terminal domain (NTD) of ~200 amino acid residues, an ATPase AAA+ domain, a Lon proteolytic domain, and a peroxisomal targeting signal type 1. Mammalian LONP2 is most related to mitochondrial LONP1 (38% identity), the second LONP member expressed in mammals. LONP1 degrades misfolded, oxidized, or unassembled proteins using its NTD to both recruit substrates and regulate allosterically the proteolytic domain. Unfortunately, the NTDs of LONP1 and LONP2 are poorly conserved, and so data on LONP1 cannot be easily extrapolated to LONP2. Thus, the nature of the substrates targeted by LONP2 and the mechanisms regulating its activity remain largely unknown. In this work, we aim to comprehensively investigate the role of LONP2 in peroxisomes. To this end, LONP2 was heterologously expressed in *E. coli* and purified using affinity and size-exclusion chromatography. The hexameric, catalytically active fraction of LONP2 was subsequently used in proteolytic assays with β-casein as a substrate. Structural analysis of LONP2 reveals distinct features compared to mitochondrial LONP1. Further studies, including the characterization of mutant variants, are currently underway.

KEYWORDS: *lonp2*, *peroxisomes*, *aaa+ atpases*

Optimization of Hermetia illucens protein recovery for potential applications in food and medicine

GABRIEL GRAÇA[1], PEDRO MOLEIRO[1], EMANUEL V. CAPELA[1], VANESSA VIEIRA[1], LUÍS C. BRANCO[2], ANA P. M. TAVARES[1], MARA G. FREIRE[1]

1. CICECO - AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, 3810-193 AVEIRO, PORTUGAL 2. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA, FACULDADE DE CIÊNCIAS E TECNOLOGIA, UNIVERSIDADE NOVA DE LISBOA, 2829-516 CAPARICA, LISBOA, PORTUGAL

The urgent need to identify alternative protein sources for improved food sustainability, due to the substantial economic and environmental costs of conventional protein sources, as well as the versatility of insect-based proteins in medicinal formulations [1] has cast a light on insects as a promising alternative [2]. Insects provide significant amounts of energy, protein, essential amino acids and micronutrients, while requiring less feed, water and land for the production of 1 Kg of edible protein compared to livestock such as poultry or cattle [3], as well as possessing meaningful amounts of other compounds of interest, such as chitin and chitosan. Accordingly, this work aimed to develop a more efficient and sustainable technology for the extraction and purification of proteins from insect flour, specifically from *Hermetia illucens* (Black soldier fly, BSF), with the ultimate goal of creating a framework that allows for the implementation of insect protein in food, medicine or other products. In this work, protein extraction was carried out with NaOH solution [3]. Initially the extraction and recovery of proteins from different BSF flours, namely defatted and non-defatted samples were evaluated. Several operational parameters were optimized by response surface methodology, including extraction time, temperature, solvent concentration and solid-to-liquid ratio. Total protein content and protein profile of the extracts were assessed by the bicinchoninic acid assay and SDS-PAGE, respectively. Based on the results obtained, it is possible to demonstrate the development of a highly efficient and more sustainable protein extraction process, surpassing previously reported findings [4,5]. Ongoing work continues to optimize operational conditions to maximize the efficiency of protein recovery.

KEYWORDS: *insects, hermetia illucens, protein extraction, sustainability*

ACKNOWLEDGMENTS:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). This study was also funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at Universidade de Aveiro, through the scope of the Agenda for Business Innovation "InsectERA" (Project no. 20 with the application C644917393-00000032). Emanuel V. Capela acknowledge FCT for the research contract 2024.08677.CEECIND.

REFERENCES:
1 Tao, X., Wu, X., Wu, H., Tu, L., Dong, M., Xiao, Y., Yu, X., & Zhong, T. (2026). A structural roadmap to gut health: A comprehensive review of edible insect chitin–protein complexes as alternative protein-derived, structure-specific prebiotics. *International Journal of Biological Macromolecules*, *354*, 151312.
2 N. M. Holden, E. P. White, Matthew. C. Lange, T. L. Oldfield, NPJ Sci Food, 2 (2018), 18.
3 Edible insects: future prospects for food and feed security. A. van. Huis, et al (Eds.), Rome, FAO, 2013.
4 L. S. Queiroz, *et al.*, Int J Biol Macromol, 186 (2021), 714–723.
5 S. Bubler, *et al.*, Helion, 2 (2016), e00218.

Extraction of bioactive proteins from insects using sustainable strategies

DANIELA B. SILVA[1], PEDRO MOLEIRO[1], NAIR CUNHA[2], EMANUEL V. CAPELA[1], ANA P.M. TAVARES[1] & MARA G. FREIRE[1]

1. CICECO - AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, 3810-193 AVEIRO, PORTUGAL; 2. THE CRICKET FARMING CO, SANTARÉM, PORTUGAL

Insects have been regarded as sustainable protein sources that can address several challenges within the biomedical field, cosmetics, and food systems vulnerability[1]. In fact, insects can be an interesting source of bioactive peptides, with potential therapeutic applications, as there are studies reporting their immunomodulatory, antidiabetic and antihypertensive effects[2]and consequently, the potential to be used as a more environmentally friendly dietary source could be beneficial for humans. On the other hand, prevention of inflammatory diseases using nutritional interventions is currently being proposed as a sustainable and cost-effective strategy to improve people's health. In this regard, finding bioactive compounds such as peptides with anti-inflammatory properties from sustainable sources (e.g., edible insects. Thus, insect proteins present high relevance not only in nutritional, but also biomedical and medicinal fields. The conventional strategies that have been commonly used for insect protein extraction usually recurs to alkaline, acid and salt solubilization, and often coupled with several pre-treatments[3]. However, such traditional methods present several drawbacks, since they considerably rely on organic solvents or highly acidic/basic conditions, and on experimental conditions that turn the processes unsustainable for the biomedical field. More efficient, milder and sustainable processes are, therefore, required. In this work, the use of more conventional and alternative/sustainable solvents, such as ionic liquids and deep eutectic solvents, was explored for the extraction of the protein fraction from cricket (*Acheta domesticus*) flour. A wide plethora of solvents were screened, and the extraction conditions were studied to obtain a cost-effective and greener process. The optimisation of the process was carried out, aiming to maximize the performance, and to obtain protein fractions with suitable features for pharmaceutical and biomedical applications. The obtained proteins functionality and characteristics were also studied, and the impact of the alternative solvents on these parameters was assessed. In summary, a high-performance and more sustainable process for the extraction of proteins was herein developed, with improved features in comparison with the extraction procedures often applied to insects, and that can deeply impact the biomedical and biotechnological field.

KEYWORDS: *insects; protein, biocompatibility*

ACKNOWLEDGMENTS:
this work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). This study was also funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at Universidade de Aveiro, through the scope of the Agenda for Business Innovation "InsectERA" (Project no. 20 with the application C644917393-00000032). Emanuel V. Capela acknowledge FCT for the research contract 2024.08677.CEECIND.

REFERENCES:
1Liceaga, A. M.; Aguilar-Toalá, J. E.; Vallejo-Cordoba, B.; González-Córdova, A. F.; Hernández-Mendoza, A. *Annu. Rev. Food Sci. Technol.* 2022, 13, 19–34.
2Rivero-Pino, F.; Rosa, T. G. la; Paz, S. M. la. *Food Funct.* 2024, 15, 2789–2798.
3Rusindo-Rodríguez, I. M.; Gutiérrez-Méndez, N.; Delgado, E.; Mateo, J.; Peralta-Pérez, M. D. R.; García-Triana, A.; Rodríguez-Valdez, L.-M. *J. Food Compos. Anal.* 2025, 147, 108070.

Reduction of gallic acid by in situ BH_3

RUI PEDRO SILVA[1,2], CRISTINA PRUDÊNCIO[1,2] & MÓNICA VIEIRA[1,2]

1. RISE-HEALTH, CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY (TBIO), E2S, POLYTECHNIC OF PORTO, R. DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200 - 072, PORTO, PORTUGAL.; 2. CHEMICAL AND BIOMOLECULES SCIENCES DEPARTMENT, SCHOOL OF HEALTH (E2S), POLYTECHNIC INSTITUTE OF PORTO, R. DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200 - 072, PORTO, PORTUGAL.

Lithium aluminum hydride ($LiAlH_4$, LAH) is one of the most widely used reducing agents in organic synthesis due to its high reactivity and broad applicability. It efficiently reduces carbonyl-containing compounds such as esters, carboxylic acids, amides, and nitriles to the corresponding alcohols or amines, whereas sodium borohydride ($NaBH_4$) is generally limited to reducing aldehydes and ketones[1] under mild conditions. The superior reactivity of LAH arises from the highly polar Al-H bond, which enables hydride transfer to less reactive carbonyl groups.[2] However, LAH presents important drawbacks: it is highly moisture-sensitive, reacts violently with water or protic solvents, and requires strictly anhydrous conditions, aprotic solvents such as THF or diethyl ether, and an inert atmosphere. On the other hand, some studies are shown a new alternative to use $NaBH_4$ in the presence of an electrophile, as iodine, generating new alternatives to the use of LAH.[3] This work presents a safer and more convenient alternative for the reduction of carboxylic acids using gallic acid as a model substrate. The methodology employs $NaBH_4$ in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$), which generates borane (BH_3) *in situ*, a much stronger reducing species capable of efficiently converting carboxylic acids into primary alcohols.[4] Compared with LAH, this approach offers milder reaction conditions, improved operational safety, and easier handling, since *in situ* generation of BH_3 avoids direct manipulation of highly reactive reducing agents while maintaining high reduction efficiency. The reduction of gallic acid is particularly significant due to its multiple hydroxyl groups and the sensitivity of the aromatic structure, making selectivity essential. Controlled addition of gallic acid to the BH_3 solution proved effective for conversion into 3,4,5-trihydroxybenzyl alcohol while minimizing possible interactions between free BF_3 and phenolic groups. Overall, this methodology represents a practical and valuable alternative for safer and milder organic reductions with good functional-group tolerance.

KEYWORDS: *gallic acid, phenolic acids, phenolic alcohols*

REFERENCES:
1 Rickborn, B.; Wuesthoff, M. T. *J. Am. Chem. Soc.* 1970, *92* (23), 6894–6904. https://doi.org/10.1021/ja00726a028.
2 Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*; Oxford University Press: Oxford, UK, 2001.
3 Simek, J. W.; Tuck, T.; Bush, K. C. *J. Chem. Educ.* 1997, *74* (1), 107. https://doi.org/10.1021/ed074p107.
4 Cho, S.-D.; Park, Y.-D.; Kim, J.-J.; Falck, J. R.; Yoon, Y.-J.. *Bull Korean Chem Soc* 2004, *25* (3), 407.

Green zero-valent iron in soil remediation and impact on Raphanus sativus L. germination, seedling growth and antioxidant system response

FILIPE FERNANDES[1,2], MIGUEL SANTOS[3], SUSANA CARVALHO[3], AGOSTINHO CRUZ[4], CRISTINA DELERUE-MATOS[1], CLARA GROSSO[1], IZABELA MICHALAK[5]

1. REQUIMTE/LAQV, INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO, INSTITUTO POLITÉCNICO DO PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, 431, 4249-015 PORTO, PORTUGAL; 2. FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO, RUA DO CAMPO ALEGRE, S/N, 4169-007 PORTO, PORTUGAL; 3. GREENUPORTO—SUSTAINABLE AGRIFOOD PRODUCTION RESEARCH CENTRE/INOV4AGRO, DEPARTAMENTO DE GEOCIÊNCIAS, AMBIENTE E ORDENAMENTO DO TERRITÓRIO (DGAOT), FACULTY OF SCIENCES, UNIVERSITY OF PORTO, RUA DA AGRÁRIA 747, 4485-646 VILA DO CONDE, PORTUGAL; 4. REQUIMTE/LAQV, ESCOLA SUPERIOR DE SAÚDE, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200-072 PORTO; 5. DEPARTMENT OF ADVANCED MATERIAL TECHNOLOGIES, FACULTY OF CHEMISTRY, WROCŁAW UNIVERSITY OF SCIENCE AND TECHNOLOGY, SMOLUCHOWSKIEGO 25, 50-372 WROCŁAW, POLAND;

Nano zero-valent iron (nZVI) is the most widely used nanoparticle for remediation in Europe and the USA. Despite this, its impacts on plant growth and soil microbiome are not fully understood [1]. To ensure the sustainable and safe use of this technology, the impacts and mechanisms must be fully elucidated. Here, the phytotoxicity of nZVI synthesized from agrifood wastes (spent coffee grounds and *Cistus ladanifer* L. post-distillation wastes) on radish ((*Raphanus sativus* L.) cv. Pharaoh obtained from WerbAna Sp. z o.o. (Warsaw, Poland)) germination and seedling growth was assessed in Petri dishes at concentrations ranging from 5 to 400 mg/L. Next, nZVI (500 mg/kg) were applied for the remediation of soils contaminated with venlafaxine and glyphosate (2 mg/kg). Afterwards, radish germination and seedling growth on the contaminated and remediated soils was assessed, along with biometric plant growth parameters, chlorophyll content, reactive oxygen species (ROS) and antioxidant system response. The nZVI did not show significant impacts on germination and plant growth, but the levels of ROS (superoxide radical and hydrogen peroxide) and the activity of some antioxidant enzymes (superoxide dismutase and ascorbate peroxidase) was slightly higher, indicating oxidative stress caused by nZVI exposure. Concerning contaminants, glyphosate caused a decrease in chlorophyll content, compared to the control plants. This study helps in understanding the potential impact of nZVI on plant growth after remediation strategies are employed.

KEYWORDS: *nzvi*, *oxidative stress*, *reactive oxygen species*

FUNDING:
This work received financial support from the PT national funds (FCT/MECI) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. The authors are grateful to MoCoffee Europe for kindly donating the coffee samples and to Naturalness Essential Oil Distillery for kindly donating the *C. ladanifer* post-distillation residues used in this study. F. F. thanks FCT/MCTES for the financial support through a PhD fellowship (2021.06806.BD, DOI 10.54499/2021.06806.BD). C. G. is thankful for her contract (2020.03436.CEECIND/CP1596/CT0008, DOI 10.54499/2020.03436.CEECIND/CP1596/CT0008) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.

REFERENCES:
1A. Galdames, L. Ruiz-Rubio, M. Orueta, M. Sánchez-Arzalluz, J.L. Vilas-Vilela, Zero-valent iron nanoparticles for soil and groundwater remediation, Int. J. Environ. Res. Public Health 17 (2020) 1–23. https://doi.org/10.3390/ijerph17165817.

Supported Ionic Liquids for Human Serum Pretreatment: Enhancing Cancer Biomarker Detection

INÊS CRUZ, FRANCISCA A. E SILVA & MARA G. FREIRE

CICECO - AVEIRO INSTITUTE OF MATERIALS, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO, 3810-193 AVEIRO, PORTUGAL

The detection of cancer biomarkers in human serum, such as Prostate-Specific Antigen (PSA) and Human Epidermal growth factor Receptor 2 (HER2), has emerged as a promising non-invasive approach for the early detection and monitoring of cancer[1]. However, the analytical sensitivity of these biomarkers is significantly hindered by the presence of highly abundant serum proteins, such as human serum albumin (HSA) and immunoglobulin G (IgG), which comprise approximately 80% of the total protein content[2]. Consequently, biomarker analysis requires sample pretreatment and detection methods that rely on expensive and time-consuming resources and protocols[2]. Supported ionic liquids (SILs) have emerged as an innovative alternative, in which ionic liquids (ILs) are immobilized on solid supports such as silica, polymers, or other substrates[3]. This combination offers the advantages of ILs, such as stability, selectivity and tunability, while overcoming some of their limitations, including handling challenges and incompatibility with some analytical techniques[4]. In this study, two different SILs, namely [Si][C_3mim]Cl and [Si][N_{3888}]Cl, were synthesized to evaluate their potential as selective sorbents for serum proteins depletion and/or biomarker enrichment. The depletion efficiencies of HSA and IgG onto SILs were obtained by size exclusion-high-performance liquid chromatography (SE-HPLC). Enzyme-linked immunosorbent assays (ELISA) were assessed to quantify biomarker levels in the pretreated serum supernatant. Results showed that [Si][C_3mim]Cl effectively isolated 83% of PSA onto its surface, while 85% of IgG and 90% of HSA remained in the pretreated serum supernatant. Conversely, [Si][N_{3888}]Cl adsorbed 38% of IgG and 72% of HSA onto the SIL, allowing for a 50% HER2 recovery in the pretreated serum supernatant. Overall, these results demonstrate the versatility of SIL-based systems as robust, tunable platforms that can enhance serum pretreatment workflows and improve the reliability of biomarker analysis for prostate and breast cancer diagnostics.

KEYWORDS: *supported ionic liquids; cancer biomarker detection; serum pretreatment*

FUNDING:
I.C. acknowledges FCT for the doctoral grant 2024.06707.BD. F.A.eS. acknowledges FCT for the researcher contract CEECIND/03076/2018/CP1559/CT0024 (DOI10.54499/CEECIND/03076/2018/CP1559/CT0024) under the Scientific Employment Stimulus – Individual Call 2018.

REFERENCES:
1Garg, S.; Sachdeva, A.; Peeters, M.; McClements, J. *ACS Sensors*. 2023, 3643–3658.
2E. Rosa, M.; S. M. Mendes, M.; Carmo, E.; P. Conde, J.; A. P. Coutinho, J.; G. Freire, M.; A. e Silva, F. *Sep. Purif. Technol.* 2023, *322*.
3Pedro, A. Q.; Coutinho, J. A. P.; Freire, M. G. *Encyclopedia of Ionic Liquids* 2019, 1–12.
4Mota-Martinez, M. T.; Brandl, P.; Hallett, J. P.; Mac Dowell, N. *Mol. Syst. Des. Eng.* 2018, *3* (3), 560–571.

Therapeutic antigen-binding fragments production by antibodies enzymatic digestion and purification using ionic liquid-based strategies

ANA MIGUEL LOUREIRO[1,2], JOÃO A.P. COUTINHO[2], EMANUEL V. CAPELA[1], MARA G. FREIRE[1]

1. CICECO – AVEIRO INSTITUTE OF MATERIALS, UNIVERSITY OF AVEIRO, PORTUGAL 2. RYA-PURIFICATION TECHNOLOGIES, ÍLHAVO, PORTUGAL

Antigen binding fragments (Fabs) emerged as highly targeted therapies with reduced side effects. The reduced dimensions, lack of crystallizable fragment (Fc), and simpler structure compared to full-length antibodies confer them better tissue penetration, higher specificity, and lower immunogenicity. Despite several advances have been made in Fab production, the downstream processing lacks standardized methodologies, relying on multiple chromatographic techniques that are difficult to scale up, are laborious and time-consuming, and require expensive affinity ligands and hazardous organic solvents[1], remaining the major contributor to their high manufacturing costs. The development of alternative purification platforms is, therefore, crucial to overcome this bottleneck and enable scalable, cost-effective production of high-quality and purity Fabs, required to meet the market demands. Ionic liquids (ILs) have been successfully applied to the extraction and partial purification of monoclonal antibodies (mAbs) from cell culture supernatants as components of aqueous biphasic systems (ABS)[2]. In this context, we propose the application of IL-based ABS as alternative platforms for the purification of Fabs produced through papain digestion of antibodies. Aiming to integrate the upstream and downstream processes, we conducted preliminary tests to ascertain the influence of ILs on papain ability to digest antibodies. Papain concentration for commercial polyclonal immunoglobulin G (IgG) digestion was optimized without the presence of IL in the digestion medium, and then, the influence of ILs in the digestion process was investigated at the best conditions. Digestion mixtures were analyzed by SE-HPLC and SDS-PAGE to assess enzymatic digestion efficiency and Fabs production. Then, IL-based ABS were studied towards the separation and purification of Fabs. These results provide new insights into ILs influence on enzymatic digestion performance and their potential application in the development of cost-effective and scalable alternative platforms for Fabs downstream processing.

KEYWORDS: *antigen binding fragments (fabs), downstream processing, ionic liquids*

ACKNOWLEDGMENTS:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). Ana M. Loureiro and Emanuel V. Capela acknowledge FCT for the PhD grant 2024.06446.BDANA (DOI 10.5449.2024.06446.BDANA), and for the research contract 2024.08677.CEECIND, respectively.

REFERENCES:
1Rodrigo G, Gruvegård M, Van Alstine JM. Antibodies. 2015;4(3):259–77. doi:10.3390/antib4030259
2Capela EV, Santiago AE, Rufino AFCS, Tavares APM, Pereira MM, Mohamadou A, et al. Green Chem. 2019; 21(20):5671–82. doi:10.1039/C9GC02733E

Mobile Application for Cardiovascular Risk Assessment

PEDRO TEIXEIRA[1], LEONOR MIRANDA[2] & MÁRIO PINTO[1]

1. ESMAD, POLYTECHNIC OF PORTO, PORTO, PORTUGAL; 2. ESS, POLYTECHNIC OF PORTO, PORTO, PORTUGAL.

Cardiovascular risk assessment plays a crucial role in the primary prevention of cardiovascular diseases, which are one of the leading causes of global morbidity and mortality. Modern society has benefited from portable and accessible means of assessing cardiovascular risk through contemporary technological trends. This effort is aligned with the scientific community's mission to reduce disability and mortality associated with cardiovascular pathologies, such as myocardial infarction or stroke, widely recognized in scientific literature. The development and incorporation of cardiovascular risk assessment tools can be considered accessible and effective strategies, both in the primary and secondary prevention of these diseases in the context of today's highly technological society, and can also contribute to a significant advance, supported by scientific evidence. This paper describes all phases of project development, covering the entire life cycle of a Web product development: project planning, state-of-the-art study, technical design process, prototyping and application development (methods and techniques, software implementation). This application may contribute to reducing cardiovascular risk and to the promotion of cardiovascular health literacy.

KEYWORDS: *cardiovascular risk assessment, mobile application; public health*

REFERENCES:
1Arvanitou, E.; Ampatzoglou, A. Journal of Systems and Software 2021, 172, 1-18.
2Biørn-Hansen, A.; Rieger, C.; et al. Empirical Software Engineering 2020, 25(4), 2997–3040.
3Nelson, M. R.; Banks, E.; et al. Med. J. Aust. 2024, 220, 482–490.

Pharmacogenomics and substance use disorders in Portugal: bridging the gap between scientific evidence and drug labelling

ANA TORRES[1], ANA RIBEIRO[1], JÉSSICA ANTÓNIO[1], MARLENE SANTOS[2]

1. SCHOOL OF HEALTH OF POLYTECHNIC OF PORTO, PORTO, PORTUGAL. 2. REQUIMTE/LAQV, ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, PORTO, PORTUGAL

Background: Substance use represent a major global public health burden requiring pharmacological interventions grounded in robust scientific evidence [(1)]. Pharmacogenomics offers a critical framework for personalised medicine by tailoring therapies to individual genetic profiles an approach particularly relevant in addiction treatment [(2)]. Objectives: To evaluate the degree of alignment between current pharmacogenomic evidence and regulatory documents, namely Summaries of Product Characteristics (SPCs) and Patient Information Leaflets (PILs), for drugs classified under the WHO N07B group and bupropion (N06AX). Methods: A structured review was conducted for drugs spanning ATC subgroups: N07BA (varenicline, cytisinicline, nicotine); N07BB (disulfiram, naltrexone, nalmefene); N07BC (methadone, buprenorphine, buprenorphine/naloxone); and bupropion (N06AX) [(3)]. SPCs and PILs were retrieved from Infomed [(4)]. Pharmacogenomic evidence was systematically sourced from CPIC guidelines and PharmGKB annotations [(5)]. For each drug, the presence, accuracy, and completeness of pharmacogenomic information in regulatory documents were assessed against these reference databases, with particular focus on clinically actionable gene–drug interactions involving variants such as *CYP2D6*, *ADH1B*, *ALDH2*, and *CHRNA5*. Results: Genetic variants including *CYP2D6*, *ADH1B*, *ALDH2*, and *CHRNA5* are established modulators of drug metabolism and dependence susceptibility across this therapeutic class. Nevertheless, SPCs and PILs for the majority of reviewed drugs contain absent or outdated pharmacogenomic information, with poor alignment to current CPIC and PharmGKB recommendations. Conclusions: Better incorporation of validated genetic data into SPCs and PILs would strengthen clinical decision-making, and support treatment personalisation, in the management of substance use disorders.

KEYWORDS: *pharmacogenomics*, *drugs of abuse*, *genetic variants*

REFERENCES:

[1] European Union Drugs Agency. EUDA, 2024. https://www.euda.europa.eu/publications/mini-guides/action-framework-for-developing-and-implementing-health-and-social-responses-to-drug-problems_pt (accessed Apr 28, 2026).

[2] Ballester, P.; Muriel, J.; Peiró, A. M. *Expert Opin. Drug Metab. Toxicol.* 2022, *18*, 261–275. https://doi.org/10.1080/17425255.2022.2085552

[3] WHO Collaborating Centre for Drug Statistics Methodology. *ATC/DDD Index 2024: N07B Drugs Used in Addictive Disorders*; WHOCC, 2024. https://atcddd.fhi.no/atc_ddd_index/?code=N07B (accessed Apr 28, 2026).

[4] Infarmed. *Prontuário Terapêutico Online: Grupo 4 Sistema Nervoso Central*; Autoridade Nacional do Medicamento e Produtos de Saúde, I.P., 2024. https://app7.infarmed.pt/infomed/ (accessed Apr 28, 2026).

[5] Whirl-Carrillo, M.; Huddart, R.; Gong, L.; Sangkuhl, K.; Thorn, C. F.; Whaley, R.; Klein, T. E. *Clin. Pharmacol. Ther.*2021, *110*, 563–572. https://doi.org/10.1002/cpt.2350

Use of Natural Compounds in Obesity: A Study on Knowledge, Attitudes, and Practices Regarding Supplements

BRUNO CASTRO[1], MÓNICA VIEIRA[1,2,3], CRISTINA PRUDÊNCIO[1,3] & SÍLVIA FERNANDES[1,4]

1. CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA (TBIO)/REDE DE INVESTIGAÇÃO EM SAÚDE (RISE-HEALTH), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO (E2S|P.PORTO), PORTO, PORTUGAL; 2. MESTRADO BIOQUÍMICA EM SAÚDE, E2S|P.PORTO, PORTO, PORTUGAL 3. ÁREA TÉCNICO-CIENTÍFICA CIÊNCIAS QUÍMICAS E DAS BIOMOLÉCULAS, E2S|P.PORTO, PORTO, PORTUGAL; 4. ÁREA TÉCNICO- CIENTÍFICA DE ANATOMIA PATOLÓGICA, CITOLÓGICA E TANATOLÓGICA, E2S|P.PORTO, PORTO, PORTUGAL.

Obesity represents a major public health challenge, being associated with high morbidity, mortality, and significant socioeconomic impact [1]. Consequently, there has been growing interest in the use of natural compounds with potential anti-obesity effects, driven by the perception that these compounds may represent a safer and more accessible alternative to conventional therapies. Considering that scientific evidence regarding their efficacy and safety remains limited [2], the present study aims to analyze the knowledge, attitudes, and practices of the Portuguese population regarding the use of natural compounds in obesity management. Following institutional ethical approval, data collection was carried out through an anonymous questionnaire developed on the Microsoft Forms platform and disseminated online through the academic community of E2S|IPP. The questionnaire includes questions designed to obtain information on sociodemographic variables, patterns of use, perceptions of efficacy and safety, level of knowledge about natural compounds, and the occurrence of adverse effects. Data were analyzed using descriptive and inferential statistics in SPSS v31 to explore possible associations between variables. To date, 683 individuals have participated in the study, the majority being female (79%), with the 45–65 age group (40%) being the most represented. The results show that 52% of participants reported having heard about natural compounds used for weight loss, while approximately 10% reported using them, with the most common being Conjugated Linoleic Acid, Berberine, and Caffeine. Among individuals who reported use, 7% indicated the occurrence of adverse effects associated with consumption. The data obtained contribute to a better understanding of population behaviors and perceptions regarding natural compounds, fostering the development of new educational strategies and promoting more informed, safe, and evidence-based use.

KEYWORDS: *obesity; natural compounds; survey; health literacy; risk perception*

REFERENCES:

1 Blüher, M. (2019). Obesity: global epidemiology and pathogenesis. Nature Reviews Endocrinology, 15(5), 288–298.

2Ríos-Hoyo, A., & Gutiérrez-Salmeán, G. (2016). New Dietary Supplements for Obesity: What We Currently Know. Current Obesity Reports, 5(2), 262–270.

Fungal-Mediated Biodegradation of Microplastics: Evaluation of Degradative Potential and Pathogenic Traits

JOEL BARBOSA[1], VIRGÍNIA CRUZ FERNANDES[1,2], JOÃO RUFO[1], MÓNICA VIEIRA[1], CRISTINA PRUDÊNCIO[1] & CLARA PEREIRA[1]

1. RISE-HEALTH, CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY RESEARCH (TBIO), ESS, POLYTECHNIC OF PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200-072, PORTO, PORTUGAL. 2. REQUIMTE/LAQV, ISEP, POLYTECHNIC OF PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA 431, 4249-015 PORTO, PORTUGAL

The increasing accumulation of plastics and microplastics in the environment represents a major global concern due to their persistence, widespread distribution, and potential impact on ecosystems and human health[1]. Microbial biodegradation has emerged as a promising and sustainable strategy for plastic remediation, particularly through the use of fungi with enzymatic degradative capacity. This project aims to test the biodegradative potential of different fungal species against the widely used microplastics Polypropylene, Polystyrene and Poly(ehylene-co-cinyl acetate), under optimized growth conditions. The fungi, *Mucor spp*, *Rhizopus spp*, *Scedosporium spp*. and *Nigrospora spp*. were specifically selected because they have never previously been investigated as plastic degraders, thereby offering the opportunity to identify novel biodegradation capabilities. *Aspergillus flavus* will also be included as a control, since this species has recognized degradative capacity against some types of microplastics[2].To identify growth conditions that maximize biodegradation, preliminary studies using *Scedosporium* spp. were performed under different glucose concentrations and medium replacement intervals. Low-glucose media combined with fortnightly medium renewal promoted the highest degradative activity and were selected for the subsequent biodegradation assays. Biodegradation will be assessed through microplastic mass loss determination, Fourier-transform infrared spectroscopy (FTIR), and Raman spectroscopy, allowing the identification of structural and chemical modifications on microplastic surfaces. Fungal adherence to microplastics will be evaluated by microscopic observation and surface colonization analysis, providing insight into biofilm formation and the role of fungal activity in the degradation process. Additionally, fungal pathogenicity-related traits, including biofilm formation and antifungal susceptibility, will be evaluated to determine whether long-term exposure to microplastics influences fungal virulence. This study may support the development of sustainable plastic biodegradation strategies and improve understanding of fungal adaptation to plastic-rich environments.

KEYWORDS: *microplastics, fungi; sustainability; biodegradation*

FUNDING:
This work received financial support from the PT national funds (FCT/MECI) through the project RISE-Health, R&D Unit (reference UID/06397/2025) and the FEDER - the European Regional Development Fund (COMPETE 2030), through the WISE project (No. 17091, ref. COMPETE2030-FEDER-00841800).

REFERENCES:
1Microplásticos: origens, efeitos e soluções europeias. Parlamento Europeu. November 22, 2018.
2Ekanayaka AH, Tibpromma S, Dai D, et al. A Review of the Fungi That Degrade Plastic. *Journal of Fungi*. 2022;8(8):772.

Antimicrobial properties of PLA scaffolds functionalized via LbL method with Manuka and multifloral honey

INÊS ALVES[1,2], RICARDO FERRAZ[1,2,3], CRISTINA PRUDÊNCIO[1,2] & JOÃO RUFO[1,2]

1. ESS, POLYTECHNIC OF PORTO, RUA DR. ANTÓNIO BERNARDINO DE ALMEIDA 400, 4200-072 PORTO, PORTUGAL. 2. RISE-HEALTH, CENTRO DE INVESTIGAÇÃO EM SAÚDE TRANSLACIONAL E BIOTECNOLOGIA MÉDICA (TBIO), ESCOLA SUPERIOR DE SAÚDE, INSTITUTO POLITÉCNICO DO PORTO, PORTO, PORTUGAL 3. LAQV-REQUIMTE, DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO, PORTO, PORTUGAL

Background: Tissue engineering and chronic wound management face obstacles due to the global rise of resistances. Natural bioactive agents, such as Manuka honey, offer a unique therapeutic profile by combining antimicrobial activity with the ability to modulate the biochemical wound environment and promote cellular regeneration. However, the seamless integration of these biological compounds into synthetic matrices, such as polylactic acid (PLA), remains a challenge. 2

Objective: This study aims to develop PLA scaffolds functionalised with honey using the Layer-by-Layer (LbL) technique and evaluate their inhibitory capacity against microbial growth. Two honey types are compared, MGO 263+/UMF10+ Manuka honey and a Portuguese multifloral honey.

Methodology: Concentrations of 15%, 20%, 25%, 30% and 35% will be tested by diluting the honey in 3M sodium acetate buffer and adjusting pH to 5.5. The method has currently been successfully optimized for the 15% concentration. The LbL process consisted of three immersion cycles, alternating with coating in the honey solutions, washing in acetate buffer and short periods of drying. As a negative control, PLA scaffolds were subjected only to the washing steps. Biological activity was assessed against *Escherichia coli* and *Staphylococcus aureus*. In the disc diffusion test, the plates were inoculated uniformly and the scaffolds arranged in quadrants, including positive controls of ciprofloxacin for *E. coli* and penicillin G for *S. aureus*. After incubation, inhibition was quantified by two perpendicular measurements of the halo. Quantification of Colony-Forming Units (CFU) was performed after recovery of the microorganisms from the scaffolds by vortexing and centrifugation in saline solution. **Results:** The study is currently in the optimization phase. Sterilization has been successfully achieved by immersing the scaffolds in 70% ethanol for 2 hours with gentle stirring, followed by vacuum drying in a Petry dish for 24 hours. Microscopic observation of the scaffolds showed a clear coating layer in functionalized samples. **Conclusions:** This research seeks to validate LbL functionalisation as a simple strategy for developing bioactive scaffolds. The present work establishes a foundation for subsequent phases involving various honey concentrations, with the goal of demonstrating a synergistic effect between the PLA scaffold and natural antimicrobial agents.

KEYWORDS: *antimicrobial*, *biomaterials*, *honey*, *scaffolds*

Blood Group Influence in Umbilical Cord Blood Immune Cell Profile

JOANA PINA[1], MELISSA OLIVEIRA[1], RICARDO FERRAZ[2] & ANDREIA GOMES[1]

1. BEBÉVIDA, CIÊNCIAS PARA A VIDA, S.A, PORTO, PORTUGAL; 2. SCHOOL OF HEALTH, POLYTECHNIC INSTITUTE OF PORTO, PORTO, PORTUGAL.

Stem cells, characterised by their remarkable regenerative potential and ability to differentiate into different cell types, hold immense promise in therapeutic applications. The umbilical cord is one source of stem cells which is easy, safe, and non-invasive for the mother and infant. BebéVida, a cryopreservation laboratory in Porto, served as the pivotal setting for this study. Beyond the therapeutic potential of stem cells, this study focuses on the intricate relationship between blood groups and the immune system. Variations in immune cell numbers across different blood groups suggest a potential influence of ABO antigens on early immune cell composition. The human immune system undergoes a remarkable journey from embryonic stages to infancy, marked by a gradual transition from reliance on innate immunity, which is genetically encoded, to the development of adaptive responses. ABO blood group antigens, determined by genetic inheritance, further shape immune characteristics, with antibodies passed from the mother providing temporary protection until the infant's immune system matures. This intricate interplay between genetic factors, maternal influence, and blood group genetics lays the foundation for lifelong immune health and disease susceptibility. As it has been demonstrated in different studies, there are potential links between ABO blood groups and cancer, cardiovascular, infectious (bacteria, viruses, and parasites), metabolic, and allergic diseases, and that is why this study was conducted, to understand the influence of blood groups on the umbilical cord blood immune cell profile. The parameters analysed were the nucleated cells, WBC, neutrophils, monocytes, lymphocytes, NRBC, and platelets. In fact, this study underscores the dual significance of umbilical cord stem cells in therapeutics and the intricate interplay between blood groups and immune system dynamics. By elucidating these relationships, we can advance personalized medicine approaches and improve our understanding of disease predisposition across diverse populations.

KEYWORDS: *umbilical cord blood, abo blood group, transfusion medicine*

REFERENCES:

1Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019 Dec 26;10(1):68. doi:10.1186/s13287-019-1165-5.

2A Cooling L. Blood Groups in Infection and Host Susceptibility. Clin Microbiol Rev. 2015 Jul;28(3):801–70. doi:10.1128/CMR.00109-14.

3Ward SE, O'Sullivan JM, O'Donnell JS. The relationship between ABO blood group, von Willebrand factor, and primary hemostasis. Blood. 2020 Dec 17;136(25):2864–74. doi:10.1182/blood.202000584.

Optimizing Melanoma Screening: AI-Based Risk Stratification for Efficient Patient Prioritization

MARGARIDA SILVA[1,2], CRISTINA PRUDÊNCIO[1,2] & PEDRO COELHO[1,3]

1. RISE-HEALTH, CENTER FOR TRANSLATIONAL HEALTH AND MEDICAL BIOTECHNOLOGY RESEARCH (TBIO), ESS, POLYTECHNIC OF PORTO, R. DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200-072, PORTO, PORTUGAL.; 2. BIOCHEMISTRY UNIT, DEPARTMENT OF BIOMEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF PORTO, ALAMEDA PROF. HERNÂNI MONTEIRO, 4200-319, PORTO, PORTUGAL; 3. CHEMICAL AND BIOMOLECULAR SCIENCES, ESS, POLYTECHNIC OF PORTO, R. DR. ANTÓNIO BERNARDINO DE ALMEIDA, 400, 4200-072, PORTO, PORTUGAL..

Background & Aim: Melanoma is a malignant neoplasm derived from melanocytes that is highly curable when detected early. However the 5-year survival rate drops from 99% to 35% when diagnosed at advanced stages [1,2]. In Portugal, constraints in the public health care system (SNS) waiting lists may compromise this critical treatment window [3]. This word presents the development of an artificial intelligence (AI)-powered clinical decision support system designed to assist dermatologists by pre-classifying melanoma risk in dermoscopic images obtained in primary healthcare settings, while optimizing patient prioritization. **Methods:** The proposed image-classification methodology employs a two-stage deep-learning computer vision pipeline implemented in Python using TensorFlow and trained on the publicly available ISIC 2020 and HAM10000 skin-lesion datasets. First, skin lesions are identified, isolated, and segmented using a U-Net architecture. Subsequently, the segmented lesion masks are classified using an EfficientNet-based model to estimate malignancy probability. The trained models were integrated into a real-time web application developed using the Reflex framework. **Results:** Preliminary results demonstrate that the U-Net architecture effectively segments lesions, reducing the influence of irrelevant artifacts during classification. Following segmentation, the EfficientNet classifier achieved an accuracy of 98.67% and an area under the receiver operating characteristic curve (AUC) of 99.8% on a test set comprising 4,500 dermoscopic images. Importantly for clinical screening applications, the model achieved a sensitivity (recall) of 0.99 for malignant lesions, corresponding to only 17 false negatives among 2,250 malignant cases. **Conclusions:** The proposed two-stage deep-learning pipeline shows potential as a clinical support tool for dermatologists. By providing automated and interpretable AI-based risk assessments, the system may assist physicians in prioritizing urgent cases with greater confidence. As a proof-of-concept, this tool has the potential to augment clinical workflows and help reduce diagnostic delays, particularly within the public healthcare system.

KEYWORDS: *melanoma, deep learning, dermatology, triage*

ACKNOWLEDGMENTS:
The authors gratefully acknowledge the Resilient P.PORTO: "IMPULSO Jovens STEAM" program's INNOVHEALTH financial support and institutional framework that made this work possible.

REFERENCES:
1Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. Cancer Biol Ther. 2019;20(11):1366-79.
2American Cancer Society. Survival Rates for Melanoma Skin Cancer by Stage. 2025.
3Entidade Reguladora da Saúde (ERS). Informação de Monitorização de Tempos de Espera no SNS (1.º Semestre 2025). Lisboa, 2025.
4Zhang P, Chaudhary D. Hybrid Deep Learning Framework for Enhanced Melanoma Detection. IEEE Trans Comput Biol Bioinform. 2025;1-11.

LIST OF ABSTRACTS

P.PORTO

ESCOLA
SUPERIOR
DE SAÚDE