I INTERNATIONAL MEETING

MOLECULES 4LIFE

20-22 September 2023 • UTAD • Vila Real • Portugal

BOOK of ABSTRACTS

Sociedade Portuguesa de Química
utad Universidade de Trás-os-Montes e Alto Douro
CQ VR Centro de Química Vila Real

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Casa Lapão

TABLE OF CONTENTS

WELCOME MESSAGE

Dear Participants,

Welcome to **I International Meeting Molecules4Life**.

Welcome to Vila Real.

The Chemistry Research Centre-Vila Real (CQ-VR) of the University of Trás-os-Montes and Alto Douro (UTAD) is pleased to host the **I International Meeting Molecules4Life**. This conference is the result of the CQ-VR commitment to stood as a centre that fosters interdisciplinary collaboration and a place where the synergy between chemists, biochemists and researchers from other fields with shared interests can flourish and contribute to innovative solutions. In this inaugural edition, Molecules4Life is dedicated to the interplay between new molecules, new molecular assemblies, and health sciences. The Meeting is organized around four topics: Molecules4…Therapeutic Applications, Molecules4…Healthy Food, Molecules4…Drug Delivery and Molecules4…Sensing and Diagnostic.

Throughout the days of this Meeting, you will have the opportunity to attend 10 inspired plenary lectures, 7 invited oral communications, 28 oral communications and 50 poster presentations. It will also be the occasion for networking moments, which will stimulate fruitful discussions and promote new scientific collaborations.

We are grateful for the sponsorship of the Portuguese Chemical Society (SPQ), the European Federation for Medicinal Chemistry and Chemical Biology (EFMC), the Vila Real City Council, and the international peer-reviewed open-access journals published by MDPI, Medical Sciences Forum and Molecules. We also thank local, national and international companies that sponsor this Meeting. Their contributions were essential.

We are excited to get on this interdisciplinary journey and we hope you truly enjoy the Meeting and take the opportunity to visit the amazing Douro region, homeland of the world-famous Port Wine.

The Chair of the Organizing Committee of the I International Meeting Molecules4Life,

Maria Manuel Oliveira

COMMITTES

Organizing Committee

- Maria Manuel Silva Oliveira - Centro de Química-Vila Real, UTAD - ***Chair***
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- Rui Campos - Centro de Química-Vila Real, INL, Portugal
- Samuel Martins Silvestre - Universidade da Beira Interior, Portugal

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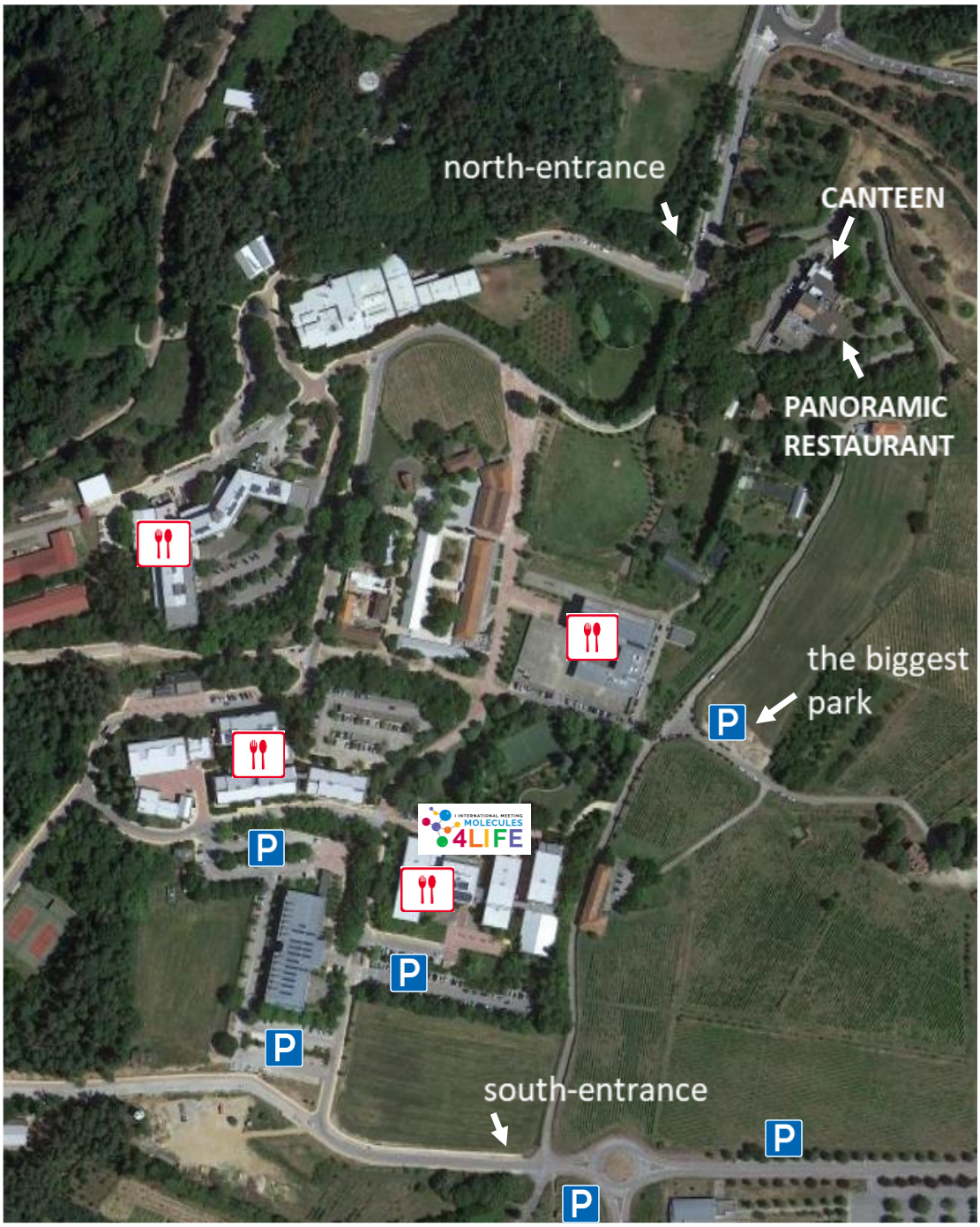
Secretariat

- Leonardo Mendes (Sociedade Portuguesa de Química - SPQ)
- Cristina Campos (Sociedade Portuguesa de Química - SPQ)

GENERAL INFORMATIONS

Location

The Meeting will take place at the University of Trás-os-Montes e Alto Douro, Escola das Ciências da Vida e do Ambiente (also known as Geosciences Building).



Registration Desk

The Registration Desk will be staffed throughout the Meeting for queries and late registrations according to the following schedule:

- Wednesday, 20th September 2023 (from 11:00 to 18:00h)
- Thursday, 21st September 2023 (from 8:30 to 17:30h)
- Friday, 22nd September 2023 (from 8:30 to 18:00h)

Sessions Location

The **Opening Ceremony**, **Closing Ceremony** and **Plenary Lectures** will be held at the Geosciences Auditorium.

Parallel Sessions will be held in the following rooms (level -1 of Geosciences Building):

- Therapeutic Applications - Auditorium 1
- Healthy Food - Auditorium 2
- Drug Delivery - Auditorium 1
- Sensing & Diagnostic - Auditorium 2
- Mixed session (Drug Delivery and Healthy Food) - Auditorium 2

Coffee Breaks

Coffee breaks will be held at Geosciences Building, close to Auditorium 1 and 2.

Lunches

- Conference lunches will be served at the Panoramic Restaurant at the University of Trás-os-Montes and Alto Douro. **Lunches are included only for senior researchers and PhD students.**
- **Undergraduate & Master student participants** can have lunch at the UTAD student´s canteen or at the several coffee-shops available on the university campus (see map). Coffee-shops sell snacks, refreshments, and light meals.

Badges

Please ensure that you wear your badge throughout the Meeting. The color coding of badges is the following:

- Red - Plenary Lecturers
- Blue - Delegates
- Green - Local Staff and Organizing Committee

Certificate of Attendance and Certificate of Presentation

Certificate of attendance or Certificate of Presentation will become available from 23rd September in your Personal Area (https://conferences.chemistry.pt) in a new menu called "Certificates". The certificates will be only accessible for those who have paid status or are exempt.

Conference Language

English is the official language of the conference.

Internet Access

UTAD provides Wi-Fi access to eduroam network. Alternatively, each participant will have access to a visitor voucher for the "guest-utad" wireless network. Vouchers can be requested at the Registration Desk.

Parking

Nearby the conference building there are several parking lots (see map). These parks are shared with the community, so the parking spaces are subject to availability.

SOCIAL PROGRAM

Welcome Drink

The Welcome Drink (included in all registration types) will take place at the conference venue in the first day of the congress (September 20th) at 18:30h, close to the poster session Hall. This get-together event will be accompanied by music played by a Student´s Musical Group (*Vibratuna* - Tuna Feminina da UTAD).

Winery Tour and Congress Dinner

The Winery Tour (by bus) and Congress Dinner will take place on the 21st of September at Favaios. The bus will depart at 17:00h from the south entrance of UTAD campus. After dinner the bus will return to this entrance, but it will park outside UTAD´s gate because the gate will be closed. On this day, delegates are strongly recommended to park their car outside the UTAD´s south entrance.

AWARDS

These prizes are awarded by the Scientific Committee and seeks to recognize the best oral and poster communication in each meeting's topic.

Eligible candidates for the oral and poster awards must be MSc or PhD students.

Best Oral Communication Award

- The prizes for the best oral presentations in all topics are sponsored by **WonderStatus**.
- The prize for the best oral presentation in *Sensors and Diagnostic* topic is a Sensit Smart device from **PalmSens**. The smallest ready-to-go potentiostat available on the market.

Best Poster Communication Award

- The prizes for the best poster presentations in all topics are sponsored by **WonderStatus**.

SCIENTIFIC INFORMATION

Oral Communications

- The oral communications are divided into:
  - ✓ **PL** | Plenary Sessions (40 minutes for presentation, plus 10 minutes for Q&A).
  - ✓ **OC** | Oral Communications (12 minutes for presentation, plus 3 minutes for Q&A).
- To guarantee that sessions run on time, speakers are kindly asked to provide the oral communication presentation files in advance, preferably during registration at the registration desk.

Posters Communications

- The poster exhibition will be located in the Geosciences Building close to Auditorium 1 and 2 (level -1).
- Posters Dimension: 90 cm (width) x 120 cm (height).
- Authors are requested to display their posters on the post panels during the first day (September 20th) and removed at the end of the conference (September 22nd).
- Material to attach posters will be made available by the organizing committee.
- Authors are requested to stay near their posters during the poster sessions.

  - ✓ **Poster Session 1 & Welcome Drink:** Wednesday 20th September, 18:30h
  - ✓ **Poster Session 2 & Coffee Break:** Thursday 21st September, 16:00-17:00h

SCIENTIFIC PROGRAM

Conference Time-Plan

Time	20th September	Time	21st September	Time	22nd September
		9.00-10.00	**PL3 -** Sensing and Diagnostic *Dr. Maria Gamella Carballo*	9.00-10.00	**PL7 -** Therapeutic Applications *Prof. Matilde Marques*
		10.00-11.00	**PL4 -** Drug Delivery *Dr. Bruno Silva*	10.00-11.00	**PL8 -** Drug Delivery *Prof. María J. Blanco-Prieto*
		11.00-11.30	*Coffee Break*	11.00-11.30	*Coffee Break*
		11.30-11.45	OC1-*SD* / OC1-*DD*	11.30-11.45	OC6-*TA* / OC5-*DD*
		11.45-12.00	OC2-*SD* / OC2-*DD*	11.45-12.00	OC7-*TA* / OC6-*DD*
		12.00-12.15	OC3-*SD* / OC3-*DD*	12.00-12.15	OC8-*TA* / OC7-*DD*
		12.15-12.30	OC4-*SD* / OC4-*DD*	12.15-12.30	OC9-*TA* / OC6-*HF*
12.00-14.00	**Registration**	12.30-14.00	*Lunch*	12.30-14.00	*Lunch*
14.00-14.30	Opening Ceremony	14.00-15.00	**PL5 -** Therapeutic Applications *Prof. Tiago Fleming Outeiro*	14.00-15.00	**PL9 -** Therapeutic Applications *Prof. Belen Vaz*
14.30-15.30	**PL1 -** Therapeutic Applications *Prof. Richard C. Hartley*	15.00-16.00	**PL6 -** Healthy Food *Prof. Justin B. Siegel*	15.00-16.00	**PL10 -** Sensing and Diagnostic *Dr. Rui Campos*
15.30-16.30	**PL2 -** Healthy Food *Prof. Pierre-Louis Teissedre*	16.00-17.00	Poster Session 2 & *Coffee Break*	16.00-16.30	*Coffee Break*
16.30-17.00	*Coffee Break*	17.00-19.30	Winery Tour / City Tour	16.30-16.45	OC10-*TA* / OC5-*SD*
17.00-17.15	OC1-*TA* / OC1-*HF*	19.30	Congress Dinner	16.45-17.00	OC11-*TA* / OC6-*SD*
17.15-17.30	OC2-*TA* / OC2-*HF*			17.00-17.15	OC12-*TA* / OC7-*SD*
17.30-17.45	OC3-*TA* / OC3-*HF*			17.15-17.30	OC13-*TA* / OC8-*SD*
17.45-18.00	OC4-*TA* / OC4-*HF*			17.30-17.45	OC14-*TA*
18.00-18.15	OC5-*TA* / OC5-*HF*			17.45	Closing Ceremony
18.30	Poster Session 1 & Welcome drink				

PL – Plenary lecture; **OC** – Oral Communication; **TA** – Therapeutic Applications; **HF** – Healthy Food; **SD** – Sensing & Diagnostic; **DD** – Drug Delivery.

SCIENTIFIC PROGRAM

Detailed Program

Time	*20th September*
12.00-14.00	**Registration**
14.00-14.30	**Opening Ceremony** ***Geosciences Auditorium*** Interventions: Prof. Dr. Emídio Gomes (UTAD Rector) Dra. Mara Minhava (CMVR Councilor) Prof. Dra. Raquel Chaves (ECVA President) Prof. Dr. Fernando Nunes (CQ-VR Director) Chaired by Maria Manuel Oliveira (UTAD)
14.30-15.30	**Plenary Lecture 1 - Therapeutic Applications** ***Geosciences Auditorium*** **Prof. Richard C. Hartley** (University of Glasgow) *Small molecules to interrogate and intervene in cellular redox* Chaired by Francisco Peixoto (UTAD)
15.30-16.30	**Plenary Lecture 2 - Healthy Food** ***Geosciences Auditorium*** **Prof. Pierre-Louis Teissedre** (University of Bordeaux) *Health potential of grape and wine phenolic compounds* Chaired by Fernando Nunes (UTAD)
16.30-17.00	**Coffee Break**

17.00-17.15	**Oral Communications**	
	Auditorium 1 Chaired by Helena Tomás (UMa) and João Rodrigues (UMa)	***Auditorium 2*** Chaired by António Inês (UTAD) and Alice Vilela (UTAD)
	OC1-*TA* - Christian Griesinger (Max Plank Institute) *Small molecules triggering transmembrane signaling and interfering with aggregation important in neurodegeneration – Invited communication*	**OC1-*HF*** - João Siopa (UTAD) *Shotgun Proteomics: a powerful tool for investigating the chemical complexity of biscuit melanoidin's*
17.15-17.30	**OC2-*TA*** - Filipe Gil (UMinho) *A game with a purpose: designing structural modifications in Polymyxin B to face multi-drug resistant bacteria*	**OC2-*HF*** - Maria Nunes (FCT NOVA) *Insect flour potential for healthy food*
17.30-17.45	**OC3-*TA*** - Tiago Santos (Inst. Hig. e Medicina Tropical) *Artemisinin extracts with ionic liquids and salts hydrotropes as a Plasmodium falciparum antimalarial strategy*	**OC3-*HF*** - Cláudia Neves (IPV) *Development of a dietary supplement designed to prepare a functional food for patients with neurodegenerative diseases*
17.45-18.00	**OC4-*TA*** - Sandra Pinto (IST) *Engineered polyurea (PURE) dendrimers kill multi-drug resistant bacteria and candida strains without affecting mammalian cells*	**OC4-*HF*** - Bruna Moreira (IPB) *Evaluation of nutritional and chemical properties of almonds (Prunus dulcis) produced in northeastern Portugal under different conditions by intercropping systems*
18.00-18.15	**OC5-*TA*** - Finbarr O'Harte (Ulster University) *Design of peptides for activation of the APJ and GLP-1 receptors and alleviation of metabolic dysfunction in diabetes*	**OC5-*HF*** - João Mota (UTAD) *Degradation of vinegar: Influence of chemical and microbiological parameters on the quality of wine vinegar*
18.30	**Poster Session 1 & Welcome drink**	

Time	21st September
9.00-10.00	**Plenary Lecture 3 - Sensing and Diagnostic** ***Geosciences Auditorium*** **Dra. Maria Gamella Carballo** (Universidad Complutense de Madrid) *Disposable affinity electrochemical biosensing platforms: towards reliable tools for food safety and personalized nutrition* Chaired by Ana Viana (FCUL)
10.00-11.00	**Plenary Lecture 4 - Drug Delivery** ***Geosciences Auditorium*** **Dr. Bruno Silva** (Swiss Federal Laboratories for Materials Science and Technology) *Advancements and challenges in nonviral gene therapeutics: a physical chemist's perspective* Chaired by Romeu Videira (REQUIMTE - LAQV)
11.00-11.30	**Coffee Break**
11.30-11.45	**Oral Communications**
	Auditorium 1 Chaired by Maria Manuel Oliveira (UTAD) **OC1-*DD*** - Ricardo Silva (IST) *Extracellular vesicles increased production by stimulation with nitric oxide-releasing polyurea biodendrimers*
	Auditorium 2 Chaired by Paula Lopes (UTAD) **OC1-*SD*** - Ana Viana (FCUL) *Electrochemical polymerization of catecholamines and catechol derivatives for optical and electrochemical biosensors* - *Invited communication*
11.45-12.00	**OC2-DD** - *Romeu Videira (REQUIMTE – LAQV)* *Exploring the therapeutic space around the mitochondrial respiratory chain to tackle chronic diseases* - *Invited communication*
	OC2-*SD* - *Isilda Amorim (UMinho)* *An electrochemical sensor based on transition metals for the detection of bioactive molecules containing phenolic groups*

12.00-12.15	**OC3-*DD*** - Sandra Nunes (UCoimbra) *Efficient models for Monte Carlo simulations applied to different biomolecules-based drug delivery systems*	**OC3-*SD*** - Ana Queijo (UTAD) *Simultaneous voltammetric determination of carbamazepine, paracetamol, and naproxen using a miniaturized electrochemical device*
12.15-12.30	**OC4-*DD*** - Carla Vitorino (UCoimbra) *Addressing hard-to-access brain tumors using a chemo-photothermal nanotechnology*	**OC4-*SD*** - Verónica Serafín (U. Complutense de Madrid) *Valorizing electrochemical multiplexing of humoral immune response biomarkers for precision chronic disease medicine*
12.30-14.00	**Lunch**	
14.00-15.00	**Plenary Lecture 5 - Therapeutic Applications** ***Geosciences Auditorium*** **Prof. Tiago Fleming Outeiro** (University of Göttingen) *Unraveling the molecular mechanisms of Parkinson's disease and related synucleinopathies* Chaired by Christian Griesinger (Max Planck Institute)	
15.00-16.00	**Plenary Lecture 6 - Healthy Food** ***Geosciences Auditorium*** **Prof. Justin B. Siegel** (University of California Davis) *Protein design and discovery to build the future of our food system* Chaired by Miguel Ribeiro (UTAD)	
16.00-17.00	**Poster Session 2 & Coffee Break**	
17.00-19.30	**Winery Tour / City Tour**	
19.30	**Congress Dinner**	

Time	22nd September	
9.00-10.00	**Plenary Lecture 7 - Therapeutic Applications** ***Geosciences Auditorium*** **Prof. Matilde Marques** (Instituto Superior Técnico) *Tackling health challenges with chemical tools: incursions into drug design and drug toxicity* Chaired by Lucinda Reis (UTAD)	
10.00-11.00	**Plenary Lecture 8 - Drug Delivery** ***Geosciences Auditorium*** **Prof. María J. Blanco-Prieto** (Universidad de Navarra) *Nanomedicine for the treatment of pediatric cancer* Chaired by Francisco Peixoto (UTAD)	
11.00-11.30	**Coffee Break**	
11.30-11.45	**Oral Communications**	
	Auditorium 1 Chaired by Christian Griesinger (Max Planck Institute) **OC6-*TA*** - Samuel Silvestre (UBI) *Oxidized arylidenesteroids with potential interest in the treatment of prostatic diseases - Invited communication*	***Auditorium 2*** Chaired by Romeu Videira (REQUIMTE - LAQV) **OC5-*DD*** - Ana Silva (REQUIMTE/LAQV - ISEP) *Oral films incorporating chestnut shells' bioactive compounds as delivery system for the prevention/treatment of oral mucositis*
11.45-12.00	**OC7-*TA*** - Andreia Fernandes (CBQ) *Chemical characterization and cellular mechanisms of mushroom polysaccharides: evaluating the potential for wound healing*	**OC6-*DD*** - Ivo Martins (UMadeira) *Synthesis of carbon dots using dendrimers as co-precursor*

12.00-12.15	**OC8-*TA*** - Mah Siau Hui (Taylor's University) *New xanthone analogues as potential lead compounds for gastric inflammatory diseases*	**OC7-*DD*** - Tânia Cova (UCoimbra) *Breaking through the blood-brain barrier: The synergy of machine learning and molecular simulations in designing cell-penetrating peptides for glioblastoma nanoparticle therapy*
12.15.12.30	**OC9-*TA*** - Maria João Queiroz (UMinho) *Synthesis of novel methyl 3-(hetero)arylthieno [3,2-b]pyridine-2-carboxylates and in vitro and in ovo antitumoral evaluation - Invited communication*	**OC6-*HF*** - Elisa Costa (UTAD) *Protein extraction from Arthrospira platensis for use in food processing*
12.30-14.00	**Lunch**	
14.00-15.00	**Plenary Lecture 9 - Therapeutic Applications** ***Geosciences Auditorium*** **Prof. Belen Vaz** (Department of Organic Chemistry, Universidad de Vigo) *Synthesis of indolobenzoazepinone scaffolds as active epigenetic modulators: challenges and opportunities* Chaired by Samuel Silvestre (UBI)	
15.00-16.00	**Plenary Lecture 10 - Sensing and Diagnostic** ***Geosciences Auditorium*** **Dr. Rui Campos** (International Iberian Nanotechnology Laboratory) *Genosensors: from small molecules to nucleic acids* Chaired by Fátima Bento (UMinho)	
16.00-16.30	**Coffee Break**	

16.30-16.45	**Oral communications**	
	Auditorium 1 Chaired by Maria João Queiroz (UMinho) and Paulo Santos (UTAD)	***Auditorium 2*** Chaired by Ana Viana (FCUL) and Rosa Rego (UTAD)
	OC10-*TA* - Mohammed Hawash (An-Najah National University) *Exploring the therapeutic potential of benzodioxol derivatives: targeting multiple biological pathways*	**OC5-*SD*** - Helena Gonçalves (Biosensor NTech Nanotechnology Services Lda) *Genetic biosensor for fast and selective SARS-CoV-2 detection - Invited communication* -*Invited communication*
16.45-17.00	**OC11-*TA*** - Adriana Cruz (IBB) *Nature-inspired anticancer polycationic core-shell dendrimers*	**OC6-*SD*** - Manuela Frasco (UCoimbra) *Engineered functional photonic materials for bioinspired sensing - Invited communication* -*Invited communication*
17.00-17.15	**OC12-*TA*** - Daniela Malafaia (UAveiro) *Chromeno[3,4-b]xanthones: on the way to a new multitarget approach for Alzheimer's disease*	**OC7-*SD*** - Dinarte Neves (BioMark@UC) *Biomimetic hydrogel for optical sensing of extracellular vesicles*
17.15-17.30	**OC13-*TA*** - Nuno Martinho (IST) *Journey to the chemical frontier of urease inhibition: molecular design guided by machine learning*	**OC8-*SD*** - Sara Gaspar (FCT UNOVA) *NO2Probe: application of a new nitrite point-of-care test in biomedicine*
17.30-17.45	**OC14-*TA*** - Tiago Coutinho (UTAD) *Assessment of safety and photo-protective capacity of three selected flavonoids*	
17.45	**Closing Ceremony** ***Geosciences Auditorium***	

LIST OF COMMUNICATIONS

Plenary Lectures

- **Therapeutic Applications**

  PL1 | Prof. Richard C. Hartley

  Small molecules to interrogate and intervene in cellular redox.

  PL5 | Prof. Tiago Fleming Outeiro

  Unravelling the molecular mechanisms of Parkinson's disease and related synucleinopathies.

  PL7 | Prof. Matilde Marques

  Tackling health challenges with chemical tools: incursions into drug design and drug toxicity.

  PL9 | Prof. Belen Vaz

  Synthesis of indolobenzoazepinone scaffolds as active epigenetic modulators: challenges and opportunities.

- **Healthy Food**

  PL2 | Prof. Pierre-Louis Teissedre

  Health potential of grape and wine phenolic compounds.

  PL6 | Prof. Justin B. Siegel

  Protein design and discovery to build the future of our food system.

- **Sensing and Diagnostic**

  PL3 | Dr. Maria Gamella Carballo

  Disposable affinity electrochemical biosensing platforms: towards reliable tools for food safety and personalized nutrition.

  PL10 | Dr. Rui Campos

  Genosensors: from small molecules to nucleic acids.

- **Drug Delivery**

  PL4 | Dr. Bruno Silva

  Advancements and challenges in nonviral gene therapeutics: A physical chemist's perspective.

  PL8 | Prof. María J. Blanco-Prieto

  Nanomedicine for the treatment of pediatric cancer.

Oral Communications

- **Therapeutic Applications**

OC1-*TA* | Christian Griesinger **(Invited communication)**

Small molecules triggering transmembrane signaling and interfering with aggregation important in neurodegeneration.

OC2-*TA* | Filipe Teixeira

A game with a purpose: designing structural modifications in Polymyxin B to face multi-drug resistant bacteria.

OC3-*TA* | Tiago Santos

Artemisinin extracts with ionic liquids and salts hydrotropes as a Plasmodium falciparum antimalarial strategy.

OC4-*TA* | Sandra Pinto

Engineered polyurea (PURE) dendrimers kill multi-drug resistant bacteria and candida strains without affecting mammalian cells.

OC5-*TA* | Finbarr O'Harte

Design of peptides for activation of the APJ and GLP-1 receptors and alleviation of metabolic dysfunction in diabetes.

OC6-*TA* | Samuel Silvestre **(Invited communication)**

Oxidized arylidenesteroids with potential interest in the treatment of prostatic diseases.

OC7-*TA* | Andreia Fernandes

Chemical characterization and cellular mechanisms of mushroom polysaccharides: evaluating the potential for wound healing.

OC8-*TA* | Nah Hui

New xanthone analogues as potential lead compounds for gastric inflammatory diseases.

OC9-*TA* | Maria João Queiroz **(Invited communication)**

Synthesis of novel methyl 3-(hetero)arylthieno[3,2-b]pyridine-2-carboxylates and in vitro and in ovo antitumoral evaluation.

OC10-*TA* | Mohammed Hawash

Exploring the therapeutic potential of benzodioxol derivatives: targeting multiple biological pathways.

OC11-*TA* | Adriana Cruz

Nature-inspired anticancer polycationic core-shell dendrimers.

OC12-*TA* | Daniela Malafaia

Chromeno[3,4-b]xanthones: on the way to a new multitarget approach for Alzheimer's disease.

OC13-*TA* | Nuno Martinho

Journey to the chemical frontier of urease inhibition: molecular design guided by machine learning.

OC14-*TA* | Tiago Coutinho

Assessment of safety and photo-protective capacity of three selected flavonoids.

- **Healthy Food**

OC1-*HF* | João Siopa

Shotgun Proteomics: a powerful tool for investigating the chemical complexity of biscuit melanoidins.

OC2-*HF* | Maria Nunes

Insect flours potential for healthy food.

OC3-*HF* | Cláudia Neves

Development of a dietary supplement designed to prepare a functional food for patients with neurodegenerative diseases.

OC4-*HF* | Bruna Moreira

Evaluation of nutritional and chemical properties of almonds (Prunus dulcis) produced in northeastern of Portugal under different conditions by intercropping systems.

OC5-*HF* | João Mota

Degradation of vinegar: influence of chemical and microbiological parameters on the quality of wine vinegar.

OC6-*HF* | Elisa Costa

Protein extraction from Arthrospira platensis for use in food processing.

- **Sensing and Diagnostic**

OC1-*SD* | Ana Viana **(Invited communication)**

Electrochemical polymerization of catecholamines and catechol derivatives for optical and electrochemical biosensors.

OC2-*SD* | Isilda Amorim

An electrochemical sensor based on transition metals for detection of bioactive molecules containing phenolic groups.

OC3-*SD* | Ana Queijo

Simultaneous voltammetric determination of carbamazepine, paracetamol and naproxen using a miniaturized electrochemical device.

OC4-*SD* | Verónica Serafín

Valorizing electrochemical multiplexing of humoral immune response biomarkers for precision chronic diseases medicine.

OC5-*SD* | Helena Gonçalves **(Invited communication)**

Genetic biosensor for fast and selective SARS-CoV-2 detection.

OC6-*SD* | Manuela Frasco **(Invited communication)**

Engineered functional photonic materials for bioinspired sensing.

OC7-*SD* | Dinarte Neves

Biomimetic hydrogel for optical sensing of extracellular vesicles.

OC8-*SD* | Sara Gaspar

NO2Probe: application of a new nitrite point-of-care test in biomedicine.

- **Drug Delivery**

OC1-*DD* | Ricardo Silva

Extracellular vesicles increased production by stimulation with nitric oxide releasing polyurea biodendrimers.

OC2-*DD* | Romeu Videira **(Invited communication)**

Exploring the therapeutic space around mitochondrial respiratory chain to tackle chronic diseases.

OC3-*DD* | Sandra Nunes

Efficient models for Monte Carlo simulations applied to different biomolecules-based drug delivery systems.

OC4-*DD* | Carla Vitorino

Addressing hard-to-access brain tumors using a chemo-photothermal nanotechnology.

OC5-*DD* | Ana Silva

Oral films incorporating chestnut shells bioactive compounds as delivery system for the prevention/treatment of oral mucositis.

OC6-*DD* | Ivo Martins

Synthesis of carbon dots using dendrimers as co-precursor.

OC7-*DD* | Tânia Cova

Breaking through the blood-brain barrier: the synergy of machine learning and molecular simulations in designing cell-penetrating peptides for glioblastoma nanoparticle therapy.

Poster Communications

- **Therapeutic Applications**

PC1-*TA* | Ana Margarida Silva

In vitro and in vivo antioxidant properties of Actinidia arguta leaves obtained by Ultrasound assisted extraction.

PC2-*TA* | Andreia Veloso

Boosting the intrinsic SOD-like activity of carbon nanomaterials.

PC3-*TA* | Beatriz Morais

Multifunctional SPIONs coated with Dextran or Leaf Extract for Theranostic Application.

PC4-*TA* | Carla Varela

Lignin extracted from Acacia wood for the development of a sustainable hair conditioner.

PC5-*TA* | Eurico Lima

GABA-modified squaraine dyes: the power of introducing amino acid units into potential PDT photosensitizers.

PC6-*TA* | Francisco Duarte

Computational design of new halogenated isoniazid derivatives - continuing the fight against tuberculosis.

PC7-*TA* | Joana Cardoso

Design and Synthesis of Siderophore-Antifungal Conjugates.

PC8-*TA* | José Teixeira

Mitochondria-targeted anti-oxidant AntiOxCIN4 improved liver steatosis and cardiac metabolic alterations in Western diet-fed mice.

PC9-*TA* | Daniela Ferreira

Mushrooms4Life: Decoding the Molecular Basis of an Anti-Cancer Small RNA Extracted from Edible Mushrooms.

PC10-*TA* | Lúcia Melo

The role of tertiary amines in chromeno[3,4-b]xanthones for Alzheimer's disease.

PC11-*TA* | Maria Inês Dias

Hepatoprotective effect of Côa Valley (Portugal) plants' extracts in a Non-Alcoholic Fatty Liver Disease (NAFLD) cell model.

PC12-*TA* | Mariana Almeida

Conjugating efflux pump inhibitors with siderophores: a novel approach to fight antibacterial resistance.

PC13-*TA* | Mpanzu Nelo

Synthesis of fluorinated carbohydrate derivatives with antibacterial potential against Gram-negative bacteria in combination with adjuvant agents.

- **Healthy Food**

PC1-*HF* | Ana Pinto

Edible coatings on grape-vine by-products infusions.

PC2-*HF* | Beatriz Marinho

Study of the sensorial and chemical properties of cookies supplemented with almond skin.

PC3-*HF* | Camelia Albu

The potential synergistic effects of melatonin on polyphenolic profile and antioxidant activity of red wines.

PC4-*HF* | Catarina Marques

Characterization of Iberian Wine Vinegar: From Flavour to Health Promoting Compounds.

PC5-*HF* | Ivo Oliveira

Health-related compounds in Portuguese almonds.

PC6-*HF* | Joana Ferreira

Health-promoting potential of pear pomace.

PC7-*HF* | José Baptista

Impact of different zones and seasons on theaflavins contents and biological properties of Azorean Camellia sinensis samples.

PC8-*HF* | Laura Pereira

Anti-biofilm and Anti-Adhesive effect of polycationic polyurea dendrimers (PURE) against Listeria monocytogenes.

PC9-*HF* | Lisete Paiva

Biological properties and L-theanine, a stress reliever amino acid, from Azorean Camellia sinensis on different plantation zones.

PC10-*HF* | Magda Semedo

Composition, nutritive value, and bioactivity of five edible algae sold in the Portuguese market.

PC11-*HF* | Man Rhee

The therapeutic effect of Korean Red Ginseng extract and Epimedium Koreanum Nakai on ulcerative colitis.

PC12-*HF* | Mónica Silva

Unpacking the impact of high-fat diet on liver antioxidant response and mitochondrial bioenergetics to microplastic-induced toxicity.

PC13-*HF* | Nuno Jorge

Plant bioactive compounds analysis for coagulation/filtration enhancement.

PC14-*HF* | Raquel Vieira

Use of natural anaesthetics for the management of fish stress with a view to sustainable production and welfare improvement.

PC15-*HF* | Sara Gonçalves

Elderberry (Sambucus nigra): A Potential Antigenotoxic Food for Promoting Health.

PC16-*HF* | Sílvia Afonso

Promoting bioactive composition and antioxidant properties of sweet cherries through biostimulant strategies.

PC17-*HF* | Sónia Martins

Functional Properties of Bioactive Compounds from Porphyridium cruentum and their Application in Food Industry.

PC18-*HF* | Teresa Pinto

Influence of Process and Chemical Composition of Chestnut on Sensory Profile and their Effect on Consumer Acceptability and Health.

PC19-*HF* | Tiago Durães

Enzymatic reduction of sugar content in sucrose-rich fruit products.

PC20-*HF* | Tiago Lopes

Effect of pre-harvest application of biostimulants on phenolic content and antioxidant activity in blueberry (Vaccinium corymbosum L.).

PC21-*HF* | Vânia Silva

Almond by-products as an alternative source of phenolic compounds and antioxidant activity.

- **Sensing and Diagnostic**

PC1-*SD* | Alexandra Costa

Green Synthesis of Luminescent Carbon Nanomaterials from Porphyridium cruentum Microalgae.

PC2-*SD* | Ana Paula Bettencourt

Sensitive determination of phenolic compounds using a Pt-GO composite modified screen-printed electrode of simple preparation.

PC3-*SD* | Belén Arjones

Aptamer-modified nanoparticles as biocatalyst.

PC4-*SD* | Catarina Costa

Evaluation of new indolenine-based squaraine dyes as potential human serum albumin fluorescent probes.

PC5-*SD* | Diogo Sousa

Advanced fluorescence characterization of biomass-derived carbon nanodots.

PC6-*SD* | Joana Martins

Protein-based photonic hydrogel for sensing microRNAs.

PC7-*SD* | Katarzyna Styszko

The profiling of PAH biomarkers in wastewater from the point of view of the risk assessment of public health exposure to hazardous chemicals in urban areas.

PC8-*SD* | Patrícia Barata

Comparative Study of Tomato Waste Carbon Dots Bioactivity: Conventional Heating vs. Microwave Irradiation.

PC9-*SD* | Sónia Ferreira

An electrochemical sensor based on carbon xerogels for detection of dopamine.

PC10-*SD* | Volodymyr Tkach

The theoretical description for aesculetin and quercetin cathodic electrochemical determination in wines.

- **Drug Delivery**

PC1-*DD* | Fátima Mendes

Development of polyester-based dendritic structures loaded with cisplatin for targeted treatment of osteosarcoma.

PC2-*DD* | Mª Almudena Acuña-Casas

Electrospinning and electrospray: potential alternatives towards improved photocatalytic systems.

PC3-*DD* | Marina Amorim

Development and characterization of the bacterial cellulose membrane impregnation process with 17-DMAG.

PC4-*DD* | Jagoda Worek

Content of microplastics in stabilized sewage sludge.

PC5-*DD* | Paula Plasencia

Chemical and bioactive evaluation of extracts produced from raspberry pruning material.

I INTERNATIONAL MEETING
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PLENARY LECTURES

Small molecules to interrogate and intervene in cellular redox

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Small molecules are widely used to sense and quantify species involved in redox biology. They can also deliver a reactive species to a specific site and so perturb the redox status. But which should you use and why? How can chemical reactivity and physicochemistry inform your choice? The lecture will explore the chemical design, advantages and limitations of strategies to localise small molecules to specific sites, focussing on mitochondrial delivery using triphenylphosphonium (TPP) cations (Figure 1). It will draw examples from our own work e.g. MitoCDNB [1] and MitoPerSulf [2]. It will distinguish between colocalization and delivery and explain the design of control compounds. It will also highlight chemoselectivity in modifications and detection (e.g. octyl itaconate [3]), and the use of TPP as an MS tag for quantification (e.g. of endogenous thioester-based acylating agents [4]).

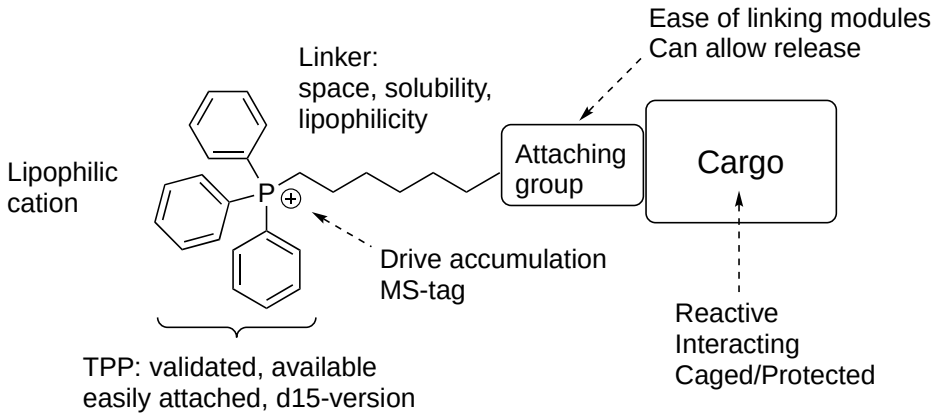

Figure 1. Generalised structure of a probe, sensor or drug targeted to the mitochondrial matrix by TPP.

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Health potential of grape and wine phenolic compounds

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Phenolic compounds or polyphenols are the most abundant and ubiquitous secondary metabolites present in the plant kingdom with more than 8000 phenolic structures currently known. These compounds play an important role in plant growth and reproduction, providing protection against biotic and abiotic stress such as pathogen and insect attack, UV radiation and wounding [1,2]. (Poly)phenols are widely distributed in the human diet mainly in plant-derived food and beverages (fruits, vegetables, nuts, seeds, herbs, spices, tea and red wine) and can influence multiple sensorial properties such as flavour and colour, and contribute to the aroma and taste e.g., astringency and bitterness [3].
Phenolic compounds are also known to have some health benefits such as a chemopreventive role toward cardiovascular, cancer, and degenerative diseases. Various epidemiological studies have shown that a regular and moderate consumption of red wine is correlated with a decreased relative risk for developing coronary heart disease. These health benefits are commonly attributed to high content of polyphenols. Many diseases and pathologies are linked, directly or indirectly, to inflammation. These include: infections, injuries, atherosclerosis, diabetes mellitus, obesity, cancer, osteo-arthritis, aged-related macular degenerescence, demyelination and the most prominent neurodegenerative diseases.
Indeed, dietary intake of (poly)phenols has been estimated to be about 1g/day [4]. Their intake is 10 times greater than that of vitamin C and 100 times that of vitamin E or the carotenoids [5]. As a result, phenolic compounds are currently receiving much attention because of their favourable health effect related to their antioxidant. To date, grape and wine polyphenols have showed to exert beneficial effects on health [6,7]. For instance, polyphenolic compounds in grapes are known to lower oxidative stress, to modulate the inflammatory cascade, to reduce the oxidation of LDL-c and to induce protection against atherothrombotic episodes including myocardial ischemia and inhibition of platelet aggregation. Most of these health effects have been ascribed to polyphenolic compounds serving as reducing agents in many biological systems by donating hydrogen, quenching singlet oxygen, acting as chelators and by trapping free radicals. Moreover, these antioxidant activities help to limit oxidation of nucleic acids, proteins, lipids, which may initiate degenerative diseases such as cancer, heart disease, dermal disorders and aging. Epidemiological studies have shown an inverse correlation between the consumption of polyphenols enriched diet and reduced risks of cardio vascular diseases (CVDs). The potential mechanisms of preventing CVDs and other chronic diseases could be related to the antioxidant activity. Actually, hypertension is also an important cardiovascular risk factors worldwide due to stroke. The importance of oxidative stress, vascular inflammation and endothelial dysfunction has to be highlighted in the development of CVDs. The knowledge of the process has provided new perspectives to elaborate dietary strategies to control the development of vascular diseases or novel "healthy" foods. Grapes composition in polyphenols and their extractability which is far from complete and typically reaching only 30–40%, depend on grape varieties, vineyard location and the technological parameters during wine making process including destemming, crushing, maceration and pressing. Therefore, grape pomace potentially constitutes a very abundant and relatively inexpensive source of a wide range of polyphenols including monomeric and oligomeric flavan-3-ols (proanthocyanidins/tannins) as well as anthocyanins (glucosides, acetylated glucosides and coumaroyl glucosides), stilbenes as well as phenolics acids and cinnamates. Moreover, it has been evaluated as a potential source of antioxidants polyphenols which could be used as nutraceuticals or food additives.

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Disposable affinity electrochemical biosensing platforms: Towards reliable tools for food safety and personalized nutrition

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Food quality is nowadays a subject of great concern to society, as the incorrect labelling of food products can represent commercial fraud as well as a health concern, especially to allergen sensitized individuals who must strictly avoid consuming them. This fact represents one of the major challenges of modern clinical nutrition, whose task is the implementation of individualized nutritional recommendations. Although there are several analytical methods available for detecting food allergens, there is still an urgent need to develop alternative methods capable of meeting some features demanded by marketization including speed, simplicity, multiplexing capability, automation, miniaturization, and reduced costs. In this context, electrochemical biosensors combine the attractive advantages of electrochemical transduction strategies with those from biosensors technology. Among them, electrochemical affinity biosensors have been used in a large number of applications, focusing either on detecting allergenic proteins or genes encoding allergenic proteins or other specific DNA fragments, providing sufficient data to evaluate food quality, as well as to determine a variety of compounds at trace levels [1].

In this presentation, attractive bioelectroanalytical tools for the determination of analytes at different molecular level will be presented. In this sense, the main features of electrochemical immunoplatforms for determining allergenic proteins in fresh and processed foods at trace levels will be highlighted. Also, attractive nucleic acid-based bioplatforms for the sensitive, selective, simple, and rapid determination of relevant animal or plant-food derived nucleic acids, using genomic fragments characteristic of coding sequences of allergenic proteins will be described. Moreover, the possibility of further simplifying the methodology and/or improving the yield of gene extraction processes through isolation of genetic information containers, such as mitochondria and chloroplasts, which contain a higher number of copies per gene [2], has also been explored. All these methodologies have been implemented onto the surface of commercial magnetic microbeads and imply amperometric transduction at screen-printed electrodes, with no need for using nanomaterials and/or complex amplification strategies to make smooth their transition from the bench to the field, in addition to being easily extended to the determination of other relevant protein targets or nucleic acids regardless of their naturally occurring variety, origin and length (from very long intact nucleic acids to degraded samples, difficult to be analyzed by conventional PCR-based methodologies).

These smart biosensing platforms can be advantageously compared with other commonly used methodologies in terms of multiplexing, even at different molecular levels, simplicity, cost, assay time and portability, which make them particularly attractive analytical tools for the implementation of affordable cost and user-friendly devices for routine determinations in field to ensure food quality and consumer protection.

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Advancements and challenges in nonviral gene therapeutics: A physical chemist's perspective

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The use of lipid-mRNA vaccines for COVID-19 has brought unprecedented success and highlighted the potential of nonviral gene therapeutics. However, this achievement is the culmination of over three decades of interdisciplinary efforts in biology, chemistry, and physics [1]. Despite this progress, the field still faces significant challenges in delivering genes to cells for the treatment of a wide range of diseases. In this talk, we will provide an overview of the main aspects of nonviral gene therapeutics, with a focus on the physical chemistry of lipid-based DNA formulations. We will highlight our own contributions to the field, specifically the use of Small-Angle X-ray Scattering (SAXS) [2] and Fluorescence Cross-Correlation Spectroscopy (FCCS) [3,4] to understand the structure and organization of these formulations. By leveraging these techniques, we can gain insight into the fundamental behavior of lipid-DNA nanoparticles and improve their design for more effective gene delivery.

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Unravelling the molecular mechanisms of Parkinson's disease and related synucleinopathies

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Protein misfolding and aggregation are common events in a wide variety of neurodegenerative disorders, such as Alzheimer's or Parkinson's disease (PD). Aging is the major known risk factor for the development of neurodegenerative diseases, but mutations in several genes are associated with familial forms. In PD, aggregation of alpha-synuclein (ASYN) in Lewy bodies and the loss of dopaminergic neurons from the substantia nigra, are typical pathological hallmarks. Our limited understanding of the molecular mechanisms underlying protein aggregation and neurodegeneration has complicated the development of novel therapeutic approaches. In our studies, we exploit different model organisms and employ diverse molecular approaches to unravel the molecular basis of neurodegenerative disorders. We are using novel cellular models where central aspects associated with ASYN dysfunction are recapitulated and we are now using powerful imaging approaches to investigate how different types of protein-protein interactions influence conformational changes in ASYN and how those relate to the initial oligomerization events associated with its toxicity. Altogether, our approaches will contribute for the development of novel strategies for therapeutic intervention in protein misfolding disorders.

Protein Design and Discovery to Build the Future of our Food System: A Journey into the Technological Advancements Transforming our Nutritional Landscape

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The global food system is currently experiencing a remarkable transformation, thanks to groundbreaking innovations in the fields of biotechnology, analytical chemistry, genomics, and data science. These emerging technologies hold the promise to revolutionize the way we produce, consume, and think about food, empowering us to not only enjoy the foods we love but also to ensure optimal health outcomes for ourselves and our planet. This talk will delve into the advancements that are shaping the future of our food system, from the creation of novel natural colors to the treatment of food-related diseases and the unlocking of the hidden potential within our food resources.

Central to this transformation is the development and application of novel protein design and discovery techniques. By harnessing the power of synthetic biology, computational methods, and genomics, researchers are unlocking new possibilities in food science, such as creating sustainable protein sources, enhancing the nutritional content of crops, and designing tailor-made proteins to cater to individual dietary needs. This talk will discuss how these innovations are offering sustainable alternatives to traditional food sources and enabling consumers to make more informed and healthier choices.

Furthermore, the synergistic advancements in data science and analytical chemistry are fostering the development of innovative solutions to some of the most pressing challenges facing our food system. This includes generating natural food colors through metabolic engineering, combatting food-driven diseases with targeted treatments, and tapping into the potential of underutilized crops to improve food security and reduce environmental impacts. By providing an in-depth exploration of these cutting-edge advancements, this talk aims to inspire and inform scientists, policymakers, and industry stakeholders as they work together to build a more sustainable, efficient, and health-focused food system for the future.

Tackling health challenges with chemical tools: incursions into drug design and drug toxicity

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The focus of our research group lies on two major research areas: the design, synthesis and evaluation of new anticancer and antibacterial drugs, and the elucidation of mechanisms of toxicity associated with xenobiotic agents of therapeutic or environmental relevance. Recent examples from both approaches will be selected for presentation and discussion.

The combined use of *in silico* tools, chemical synthesis and proof-of-concept biochemical and biological testing will be presented to describe the targeting of epigenetic pathways with relevance to cancer initiation and progression, and of glycolysis enzymes overexpressed in cancer cells. Our advances in exploring the potential of small organic molecules in cancer immunotherapy and in tackling novel targets against methicillin-resistant *Staphyloccocus aureus* (MRSA) will also be discussed.

The use of mass spectrometry-based Omics approaches to elucidate systemic effects of drugs on major biochemical pathways will be addressed in the context of the proposed repurposing of the asthma drug montelukast.

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Nanomedicine for the treatment of pediatric cancer

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This talk is about the use of nanomedicines for pediatric cancer in the light of the dismal current situation in childhood cancer management. Compared to the situation with adults, agencies, governments and institutions have pointed to an alarming gap concerning the arrival of novel approaches in children. Indeed, chemotherapy protocols for childhood cancer are still problematic due to the high toxicity associated with chemotherapeutic agents and incorrect dosing extrapolated from adults. Childhood cancers, like adult cancers, have to receive special attention with a view to developing novel therapies that are fit for purpose [1].

Osteosarcoma is the most frequent primary bone tumor in the pediatric population and one of the most frequent causes of pediatric cancer death. Due to its aggressive local growth pattern and its high propensity to metastasize, mainly to the lungs, it represents one of the leading causes of pediatric cancer death [2].

Osteosarcoma treatment is based on a neo-adjuvant multiagent chemotherapy followed by a surgical resection and adjuvant chemotherapy. Methotrexate, doxorubicin, ifosfamide and cisplatin combinations have shown the highest activity treating the non-metastatic disease [3].

During the past decades the advances in osteosarcoma treatment have been scarce without a significant improvement in survival. For instance, although doxorubicin is used as a first-line antineoplasic agent in the treatment of osteosarcoma, its narrow therapeutic window, severe adverse effects and the development of multidrug resistances have led researchers to investigate alternative forms of administering doxorubicine for cancer therapy [4].

The focus of this talk will be to discuss the potential of nanosystems loaded with edelfosine [5], a new antitumor agent, that has shown efficacy in several cancer cell lines [6,7], in the treatment of osteosarcoma [8,9].

Acknowledgments: We are indebted to all our patients and families.

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Synthesis of indolobenzoazepinone scaffolds as active epigenetic modulators: challenges and opportunities

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Paullones constitute an important class of compounds featuring a broad range of biological activities and notably antitumor action [1]. These molecules share a common indolobenzoazepinone scaffold, with variations in the substitution pattern. Due to the increasing importance of these compounds in medicinal chemistry, an efficient synthetic strategy for the preparation of this scaffold would be of high interest. Along these lines, we have recently reported the use of a Pd-driven cascade process that allows the regioselective preparation of benzofuran-, indole- and 1H-isochromen-1-imine-type derivatives through a heterocyclization-oxidative Heck cascade transformation [2]. The application of the intramolecular variant of this approach has also been applied to the preparation of differently substituted paullones featuring an exocyclic olefin at the C7 position of the benzazepinone ring. We propose the application of a one-pot protocol to efficiently prepare this scaffold from simple reactants, following a Sonogashira-heterocyclization-Heck coupling cascade process to obtain indolobenzoazepinones in a straightforward fashion.

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Genosensors: from small molecules to nucleic acids

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Biosensors are analytical devices that measure/detect biological or chemical substances by generating a signal proportional to the concentration of analyte. They are composed of three parts: a biorecognition layer, a transducer and a signal processor. Genosensors are biosensors whose biorecognition layer is composed of nucleic acids; these can be DNA, RNA, or an oligonucleotide mimic. Interest in this type of biosensor has increased exponentially in the last decade, mainly due to their versatility with applications in the environmental, food, health and drug discovery industries.
This talk will focus on three different transducers (field-effect transistors [1], electrochemiluminescent [2,3] and photoelectrochemical [4]) that can be used for the detection of various analytes, including small molecules and nucleic acids.
DNA hybridisation was detected using all three transducers. An electrolyte-gated field-effect transistor, with a two-dimensional channel made of a single graphene layer, was developed and was able to achieve label-free detection of DNA hybridisation down to attomolar levels [1]. Electrochemiluminescence (ECL) and photoelectrochemistry (PEC) were used to detect micro-RNAs associated with cancer [3,4]. In both assays, the excitation and readout of the signal are independent: an electrochemical stimulation followed by an optical readout for ECL, and the reverse for PEC. A 'sandwich' approach is incorporated, consisting of a biotinylated capture probe immobilized on streptavidin-functionalized surfaces and a detection probe labelled. Micro-RNAs were quantitatively measured in medical samples from prostate cancer patients by a low-cost electrochemical setup equipped using a LED and without the need for additional PCR or other amplification techniques.
Electrochemiluminescence was the approach chosen to develop a proof-of-concept assay for the detection and quantification of small molecules based on aptamer recognition [2]. Testosterone was used as the model small molecule and the assay showed selectivity and sensitivity, which opens a new avenue for the development of reliable and robust ECL biosensor assays for biochemical analysis.

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Small molecules triggering transmembrane signaling and interfering with aggregation important in neurodegeneration

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The switching of the two components system sensor CitA is effected by citrate. Transmembrane signaling due to citrate binding will be discussed [1]. Further, we have studied the process of aggregation of α-synuclein on membranes *in vitro* and identified key time points in the aggregation process, that enable targeted isolation of a so called intermediate I and the fibrillar endpoint [2]. Intermediate I has the characteristics of a toxic oligomer. In addition, we determined the structure of anle138b, a clinical drug candidate [3] bound to fibrils that were grown in the presence of lipids [4] that are doped with anle138b [5]. Comparison of the binding site of anle138b with compounds that bind even tighter to α-synuclein fibrils and might therefore be useful for diagnostics will be discussed.

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A Game with a Purpose: Designing Structural Modifications in Polymyxin B to Face Multi-drug Resistant Bacteria

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The spread of multidrug-resistant (MDR) microorganisms is a major health threat, as MDR strains of commonly found bacteria can transform everyday small infections into life-threatening situations. Indeed, MDR infections not only pose an immediate health and life risk, but also increased morbidity for survivors and increased healthcare and social security costs [1]. As of yet, polymyxin B (Figure 1) has been one of the "last resort" drugs in the fight against MDR infections, but resistance to this peptide has already been reported [2]. Thus, computational tools allowing the molecular design of new molecules with promising antimicrobial activity towards MDR strains are of great interest, given the urge to develop new antimicrobial drugs targeting these organisms.
In this work, we report our latest progresses aimed at designing novel polymyxin derivatives by exploring concurrent Quantitative Structure-Activity Relationship (QSAR) models [3] targeting multiple aspects of the molecule's biological activity. In particular, we focused on novel algorithms targeting the automatic exploration of the synthetically available structural modifications. The current results suggest a relatively low number of "activity hotspots", the exploration of which may shred a new light on the results from previous SAR and QSAR studies. The ultimate goal is to be able to re-design the polymyxin B structural activity puzzle by bringing key new pieces into play that will revive this last-resort antibiotic.

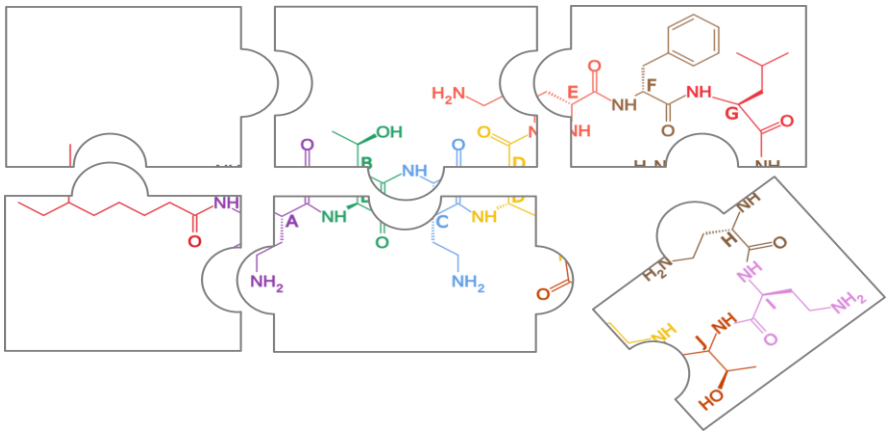

Figure 1. The structural formula of Polymyxin B1: a puzzle we need to re-design in order to face the challenges of MDR bacteria.

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Artemisinin extracts with ionic liquids and salts hydrotropes as a *Plasmodium falciparum* antimalarial strategy

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Over the last two decades, *Plasmodium falciparum* (*P. falciparum*) malaria treatment has improved significantly due to the development of therapeutics derived from artemisinin, a compound extracted from *Artemisia annua* L. leaves [1,2]. Despite the rapid parasite clearance of artemisinin against all *P. falciparum* blood stages, its biological activity can become compromised by low solubility in water due to the lack of any functional group that would provide solubilizing properties [2-4]. In addition, toxic volatile organic solvents are often used in artemisinin extraction [5]. Therefore, an efficient, sustainable, and environmentally friendly extraction procedure that preserves artemisinin's bioactivity while increasing its solubility in water is required [4,5]. Bearing this in mind, we evaluated the antimalarial activity of two artemisinin extracts in aqueous solutions of cholinium-based ionic liquids (cholinium salicylate I and cholinium salicylate II) and the corresponding salt (sodium salicylate), all of which with hydrotropic properties. We also evaluated the antimalarial activity of artemisinin extract in dichloromethane, commercial artemisinin, and the aqueous solutions of hydrotropes. We performed an antimalarial activity assay by fluorimetry using the Whole-Cell SYBR Green I method to determine the half of the maximum inhibition effect (IC50) values in the presence of unsynchronized cultures of *P. falciparum* artemisinin-sensitive 3D7 strain, with concentrations ranging from 0.1-0.001 µg/mL and to a haemotoxicity assessment by spectrophotometry, with concentrations ranging from 10-0.001 µg/mL. We observed that sodium salicylate, cholinium salicylate I, cholinium salicylate II, dichloromethane extracts, and commercial artemisinin presented IC50 values of 0.0027 ± 0.0005 µg/mL, 0.0034 ± 0.0009 µg/mL, 0.0128 ± 0.0038 µg/mL, 0.0200 ± 0.0078 µg/mL, and 0.0066 ± 0.0025 µg/mL, respectively, whereas the aqueous solutions of hydrotropes did not present biological activity. Sodium salicylate and cholinium salicylate extracts are not hemotoxic at the closest dose of the IC50 values. The salicylate salts extracts might bring new avenues for the development of potential antimalarial drugs.

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Engineered polyurea (PURE) dendrimers kill multi-drug resistant bacteria and candida strains without affecting mammalian cells

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Infectious diseases caused are a disturbing societal burden that puts in risk millions of people worldwide. This is a problem of major concern and utmost priority especially due to the emergence of novel resistant pathogens and infectious diseases. In this sense, the development of new antimicrobial agents with novel mechanisms of action is of critical importance. In 2017, the World Health Organization (WHO) made an appeal to the scientific community, stating that R&D strategies should focus on the discovery and development of new antimicrobial agents.

Considering this scenario, we developed engineered polyurea (PURE) dendrimers, densely positive charged core-shell nanoparticles that mimic antimicrobial peptides, as a novel class of antimicrobial agents. Novel cationic core-shell PURE dendrimers were synthetized following a sustainable protocol. The antimicrobial capacity (MIC and MBC values and colony count kinetic assay) was tested against several multidrug resistant (MDR) bacteria and candida strains. Also, *in vivo* assays were carried out using the insect model *G. mellonella* infected with lethal doses of MDR bacteria.

At shorter incubation time we observed that engineered PURE dendrimers can induce a 5 to 7 log reduction in colony-forming-units of several relevant bacteria and Candida strains. Importantly, at the same inhibitory concentration, we did not detect any hemolytic activity.

Cationic core-shell PURE dendrimers were able to improve the survival of *G. mellonella* after pre-injection with lethal doses of *S. aureus* and *P. aeruginosa* without causing additional toxicity.

Our results also point towards a fast-killing mechanism triggered by possible interactions with bacterial lipid membranes which were further confirmed by silico coarse-grained molecular dynamics (CGMD) simulations. These simulations suggested that the initial interactions are associated with alterations in the curvature of a model microbial cells membrane, which may lead to pores formation and bacteria death.

In a scenario of increasing resistant pathogens and infectious diseases, cationic core-shell PURE dendrimers emerge has a step forward in the development of effective and reliable antimicrobial agent.

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Design of peptides for activation of the APJ and GLP-1 receptors and alleviation of metabolic dysfunction in diabetes

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Background: Current diabetes therapies often fail to reach target glycaemic control. The adipokine apelin derived from processing of the precursor preproapelin, exists in multiple truncated isoforms. Novel stable apelin-13 peptide analogues acting via their cognate APJ receptor, have shown promising acute antidiabetic effects in high-fat fed diet induced obese (DIO) mice, as well as in suppressing appetite and reducing food intake in diet restricted trained healthy mice. In addition, apelin analogues are known to be cardioprotective, display anti-hypertensive actions, as well as reducing vascular inflammation. The actions of apelin analogues are thus complementary to those of incretin mimetics (GLP-1 analogues) which are commonly used to treat subjects with Type 2 diabetes mellitus.
Aim: This study compared the efficacy of apelin-13 related peptide analogues versus incretin mimetics in animal models of diabetes, as well as examining their complementary actions.
Methods: Various acylated and non-acylated peptide analogues of apelin-13 were produced and tested for anti-diabetic and anti-obesity actions in different models of metabolic disease including high-fat fed DIO mice and leptin receptor-deficient diabetic *db/db* mice (n=8). Comparative studies were performed using twice daily injections with saline (controls), apelin-13 analogues, incretin mimetics exendin-4(1-39) or liraglutide in acute and chronic studies. Progressive changes in glycaemic control, glycated haemoglobin (HbA1c) and plasma insulin were monitored in chronic (21-day) studies. To further examine the positive effects of apelin analogues on pancreatic responses we investigated their potential benefits on islet cell apoptosis, proliferation and transdifferentiation using $Ins1^{Cre/+}$;Rosa26-eYFP transgenic mice following diabetes induction with either streptozotocin or high-fat feeding.
Results: Apelin and incretin analogue treatment significantly improved both oral and intraperitoneal glucose tolerance, accompanied by enhanced insulin responses compared with saline-treated control *db/db* mice. Apelin-13 analogues were superior to incretin mimetics in markedly lowering (34% reduction) circulating plasma triglycerides. Immunocytochemistry studies revealed that apelin analogues unlike both incretin mimetics reduced pancreatic α-cell area, however all peptide treatments enhanced pancreatic insulin content. Apelin-13 analogues effectively reduced β- to α-cell transdifferentiation and decreased β-cell apoptosis and α-cell proliferation in both diabetic models.
Conclusion: Overall, apelin-13 analogues, induced similar and sometimes more effective metabolic improvements than incretin mimetics in *db/db* mice, providing a viable and effective alternative approach for counteracting metabolic dysfunction in diabetes. Studies have shown that peptide derived GLP-1 and APJ receptor agonists have complementary bioactivities which help alleviate metabolic dysfunction.

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Oxidized arylidenesteroids with potential interest in the treatment of prostatic diseases

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Benign prostatic hyperplasia (BPH) and prostatic cancer (PCa) are the most prevalent prostatic diseases and have high prevalence and impact in society nowadays. Androgens are produced in steroidogenic tissues and bind to the androgen receptor (AR), initiating transcription which in turn results in the synthesis of prostate-specific proteins, as well as in cell proliferation, which is the pathophysiological basis for BPH and PC. Several therapeutic approaches are being used for these conditions, however, these have lower efficacy than the desired and have debilitating side effects. These facts are motivating researchers and industries to the development of improved therapies. Steroids and their oxidation products are widely distributed in living organisms, have relevant bioactivities and are important intermediates for the synthesis of many biologically active molecules. In this context it is important to mention that several steroids are being used in the treatment of the most common prostatic diseases, such as finasteride and abiraterone acetate [1]. This presentation will focus on our recent findings on oxidized steroidal derivatives with potential interest in the treatment of the most prevalent prostatic diseases. Interestingly, among other modified steroidal derivatives [2], arylidene-Δ^4-3,6-dione steroids have relevant 5α-reductase inhibitory effects and antiproliferative effects in tumoral prostatic cell lines. In addition, of these compounds, 16*E*-(2',4'-dichlorobenzylidene)-androst-4-ene-3,6,17-trione (Figure 1) led to apoptosis of androgen-dependent LNCaP cells.

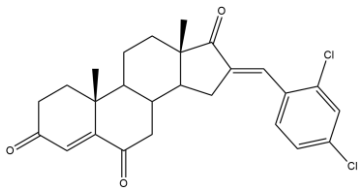

Figure 1. Chemical structure of 16*E*-(2',4'-dichlorobenzylidene)-androst-4-ene-3,6,17-trione.

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Chemical Characterization and Cellular Mechanisms of Mushroom Polysaccharides: Evaluating the Potential for Wound Healing

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Mushroom polysaccharides have been studied for their numerous bioactive properties, including their ability to modulate immune cells and wound healing process. These complex carbohydrates revealed the ability to stimulate the proliferation of fibroblasts and enhance the production of collagen. Furthermore, studies have shown that polysaccharides can modulate immune cells such as macrophages, natural killer cells, and T-cells, decreasing inflammation in the wound site. These properties make mushroom polysaccharides potential candidates for therapeutic interventions [1,2]. Identifying their chemical and structural features is crucial to understand their biological activities. With that purpose, in this work, water and alkaline-soluble polysaccharides were isolated from the biomass of three different mushroom species (*Coriolus versicolor* (CV), *Pleurotus ostreatus* (PO), and *Hericium erinaceus* (HE)) using different degrees of purification (resulting in five fractions). In all extractions the mushroom powder was submitted to hot water extraction for two hours, followed by centrifugation to collect the water-soluble and insoluble fractions. Extraction A did not have any purification step. In extraction B and C ethanol was used to precipitate the water-soluble polysaccharides, however, in extraction C was added a pre-treatment step with ethanol. In extraction D, the insoluble fraction of fraction C was submitted to an alkaline treatment followed by ethanol precipitation. All the resultant fractions were freeze-dried and analyzed. Chemical composition assays were performed to understand their α- and β-glucans content, monosaccharide composition, fatty acids, total protein, and fiber. The molecular weight (MW) distribution was also compared through HPLC–SEC. The results suggested that different extraction and purification processes showed different chemical compositions. Compared to extraction A, the extractions B with ethanol purification showed to decrease the protein content: approximately 4.4% to 0.6% (CV), 4.1% to 0,9% (PO), and 4.8% to 2.0% (HE), not affecting other parameters such as total glucans or MW. In extraction C, a previous treatment with ethanol did not show statistical differences compared to extraction B. The alkaline fraction also showed similar values of protein content between the three species (approximately 1.3, 1.4, and 1.6%, respectively). The alkaline-soluble fraction showed a very distinct polysaccharide profile, mainly composed of low-MW polysaccharides (>6 kDa), while the other fractions showed a very similar MW distribution. In the two fractions obtained from extraction A, a similar fatty acid profile was identified, and the presence of palmitic, oleic, and linoleic acid was detected. As expected, the water-soluble fraction showed a decrease in the amount of these fatty acids, since lipids are poorly soluble in water. Different from other wild mushrooms, commercial mushrooms have shown higher levels of α-glucans than β-glucans, which was corroborated by this study. The results indicated that the three species possess an α-glucan content higher than 50 g/100 g. This is an opportunity to evaluate the role of α-glucans in biological processes, once they are less studied than *β*-glucans. Considering these results, *in vitro* and *in vivo* experiments will be performed to evaluate their antioxidant and anti-aging potential. Finally, the immunomodulatory ability of these molecules also will be assessed on keratinocytes, macrophage, and fibroblast cell lines. Overall, this work intends to shed some light on the promising prospects of mushroom polysaccharides as a novel therapeutic approach for the treatment of wounds.

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New Xanthone Analogues as Potential Lead Compounds for Gastric Inflammatory Diseases

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An elevated level of reactive oxygen species (ROS) often results in oxidative stress, subsequently leads to the initiation and progression of gastric inflammatory diseases (GID). Compounds that could upregulate the intrinsic antioxidant Nrf2/ARE signaling pathway has been claimed to be a promising approach to treat GID. Xanthone, a class of natural product, is a potential candidate due to its wide range of biological activities including antioxidant, anti-inflammatory, and anticancer [1,2]. Their activities are relied primarily on the types of substituent group and their relative position attached to the xanthone analogue [3]. Thus, this study aimed to synthesize new xanthone analogues from 3-hydroxyxanthone for their structure-activity relationship on intracellular ROS inhibition level. The analogues were purified by chromatography techniques and structurally characterized via NMR, MS and FTIR spectroscopy. The antioxidant activity of the xanthone analogues was then evaluated by measuring the intracellular levels of ROS using the fluorimetric 2',7'-dichlorofluorescein diacetate assay in H_2O_2-induced oxidative damage model using SNU-1 gastric cells. Modulation of the gene and protein expression of Nrf2 and downstream proteins were assessed using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot, respectively. Remarkably, the xanthone analogues inhibited the H_2O_2-induced generation of ROS and restored the levels to that of negative control. Moreover, its activity was comparable to sulforaphane, an established Nrf2 activator. Further molecular investigations showed concentration-dependent increases in the mRNA expression of Nrf2 and downstream antioxidant enzymes HO-1, NQO1, SOD, and CAT. Similar results were obtained for the protein expression of these enzymes even though no marked increase was observed for Nrf2. Thus, attenuation in the generation of ROS by the xanthone analogues was attributed to the antioxidant action of HO-1, NQO1, SOD and CAT. In summary, xanthone analogues possessed strong intracellular antioxidant activities and thus are potential lead compounds for GID. Further in vivo studies are recommended to evaluate thephysiological relevance of present findings.

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Synthesis of novel methyl 3-(hetero)arylthieno[3,2-*b*]pyridine-2-carboxylates and *in vitro* and *in ovo* antitumoral evaluation

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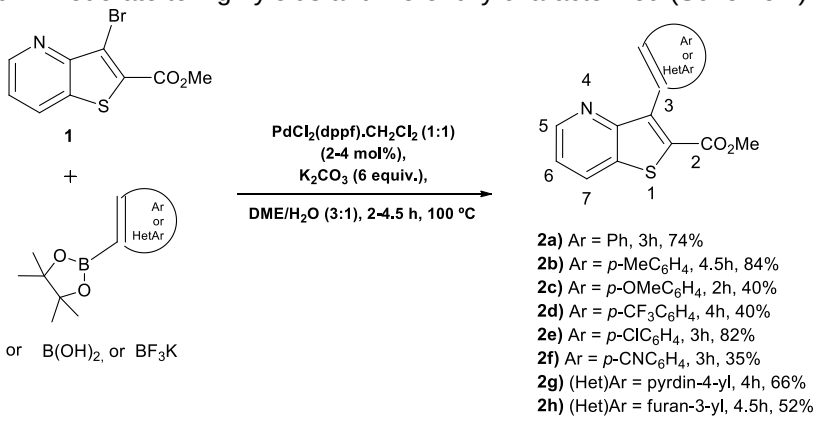
The most aggressive, invasive and with poor prognosis type of breast cancer is the triple negative breast cancer (TNBC), which is immunohistochemically characterized by the lack of estrogen receptor, progesterone receptor and absence of human epidermal growth factor receptor 2 (HER2) overexpression [1].
In this work, using the Pd-catalyzed Suzuki-Miyaura cross-coupling of the methyl 3-bromothieno[3,2-*b*]pyridine-2-carboxylate **1**, also prepared, with (hetero)aryl pinacol boranes, trifluoro potassium boronate salts or boronic acids, novel methyl 3-(hetero)arylthieno[3,2-*b*]pyridine-2-carboxylates **2a**-**2h** were synthesized in moderate to high yields and were fully characterized (Scheme 1).



2a) Ar = Ph, 3h, 74%
2b) Ar = *p*-MeC_6H_4, 4.5h, 84%
2c) Ar = *p*-$OMeC_6H_4$, 2h, 40%
2d) Ar = *p*-$CF_3C_6H_4$, 4h, 40%
2e) Ar = *p*-ClC_6H_4, 3h, 82%
2f) Ar = *p*-CNC_6H_4, 3h, 35%
2g) (Het)Ar = pyrdin-4-yl, 4h, 66%
2h) (Het)Ar = furan-3-yl, 4.5h, 52%

Scheme 1. Synthesis of Suzuki-Miyaura cross-coupling products **2a**-**2h**

The antitumoral potential of the compounds prepared was evaluated in two triple negative breast cancer (TNBC) cell lines-MDA-MB-231 and MDA-MB-468, by sulforhodamine B assay. Their effects on the non-tumorigenic MCF-12A cells were also evaluated. The results demonstrated that compounds **2e**, **2f**, **2h** caused growth inhibition in both TNBC cell lines, with little or no effect against the non-tumorigenic cells. Compound **2e** was the most promising against MDA-MB-231 cell line both *in vitro* (by decreasing viable cell number, cell proliferation and interfering with the cell cycle profile) and *in ovo* using the chick chorioallantoic membrane (CAM) model, by reducing grafted MDA-MB-231 tumor size.
This work highlights the antitumor potential of methyl 3-arylthieno[3,2-*b*]pyridine-2-carboxylates obtained by Suzuki-Miyaura C-C coupling, against aggressive TNBC [2].

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Exploring the Therapeutic Potential of Benzodioxol Derivatives: Targeting Multiple Biological Pathways

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This project aimed to synthesize Novel series of Benzodioxol derivatives, categorized into three distinct groups: benzodiazepine, aryl acetate, and acetic acid. The purpose was to assess their biological activities on multiple targets. The analogues were then evaluated in vitro and in vivo for their effects on AMPA receptor, COX, lipase, α-amylase, DPPH enzymes, and various cancer cell lines. Among the different groups, the benzodiazepine structures demonstrated activity on AMPA receptors [1, 2]. Aryl acetate derivatives exhibited significant potency against COX2 enzymes, while two compounds from the acetic acid group selectively targeted COX2 over COX1 enzyme [3] . Furthermore, three compounds from the acetic acid group and one compound from the acetate group displayed potent activity against α-amylase enzyme, with IC50 values lower than those of the Acarbose positive control [4]. Additionally, one compound exhibited strong cytotoxic activity against the MCF7 cancer cell line. However, these compounds demonstrated weak or negligible activity on the lipase enzyme [5]. To identify the interactions between Benzodiazepine compounds and AMPA receptors, as well as between the aryl acetic acid group and α-amylase enzyme, molecular docking studies were conducted. Theoretical predictions of bioavailability, determined through Molinspiration calculation and Lipinski's rule of five, correlated well with experimental verification. Overall, this project has provided potential drug candidates targeting AMPA receptors for various neurological diseases (benzodiazepine group), a promising group of Benzodioxole compounds as novel Non-steroidal anti-inflammatory agents against COX enzymes, and promising compounds for the treatment of diabetes (as shown in Figure 1).

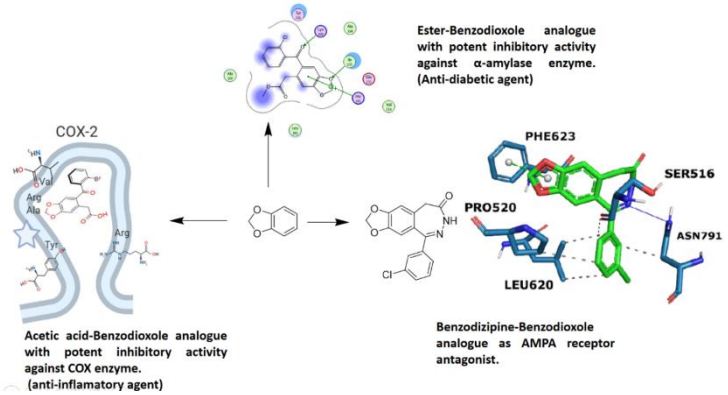

Figure 1. The promising compounds of benzodioxol on different biological targets.

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Nature-inspired anticancer polycationic core-shell dendrimers

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Despite the many efforts made in the last years to find new anticancer drugs, some challenging critical issues persist in current therapies, such as chemoresistance and off-target effects. Considering the overall modifications that occur in a cancer cell, like altered plasmatic membrane and lipids metabolism (which includes the presence of negatively charged phospholipids in the outer leaflet of the membrane), one possible approach is the design of therapeutics agents targeting specifically the cancer cells membranes.

Cationic polymers are synthetic mimics of antimicrobial peptides (SMAMPs) and, due to strong electrostatic interactions with negatively charged membranes, are reported as potential anticancer drugs [1]. Herein, we demonstrate the intrinsic anticancer activity of novel core-shell polycationic dendrimers, PURE-PEI [2] and PURE-CEI, prepared from polyurea dendrimers precursors [3]. Our studies point to an affinity of both dendrimers towards the cancer cell membrane, interacting with the negatively charged groups present in the polymers surface. Furthermore, we also observed a specific localization at mitochondrial level leading us to hypothesize that they can activate the intrinsic apoptotic pathway.

Importantly, these pharmadendrimers are synthetized using sustainable protocols and have shown unique and rather attractive properties as potential nanotherapeutics.

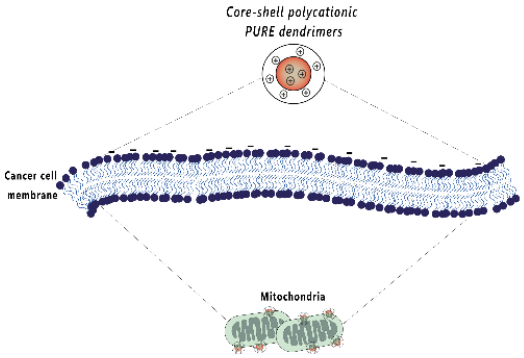

Figure 1. Core-shell polycationic PURE dendrimers interacting with the cancer cell membrane and targeting mitochondria.

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Chromeno[3,4-*b*]xanthones: on the way to a new multitarget approach for Alzheimer's disease

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The Alzheimer's disease (AD) is a complex multifactorial neurodegenerative disorder, mainly characterized for the progressive and unremitting memory loss and cognitive, motor, and functional capacity [1]. To date, there is no cure or prophylaxis for this neurological disorder, as the clinically available drugs only provide limited symptomatic treatment and do not alter the course of the disease [2]. In fact, since 2003, only two drugs have been approved for AD by the fast-tracked approval of Food and Drug Administration (FDA) [3,4]. However, due to its high cost (an estimated value of up to $26.500 per year), many experts believe that the number of people that will be able to get the drugs will be extremely limited, particularly low-income and middle-income countries (LMICs), with under-resourced public health systems [5]. For this reason, there has been a worldwide effort to develop an effective and more affordable therapy for AD, such as small molecules, which are cheaper, more convenient to administer and widely accessible. To address this issue, in 2021 we disclosed a novel class of multifunctional chromeno[3,4-*b*]xanthone derivatives [6]. Herein, we describe the lead optimization effort to establish a complete profile of these compounds *in vitro*, including design, synthesis, anticholinesterase and antiaggregating properties, molecular docking studies and saturation-transfer difference (STD) binding epitopes, cytotoxicity in human neuroblastoma cell line (SH-SY5Y) and blood–brain barrier (BBB) permeability prediction.

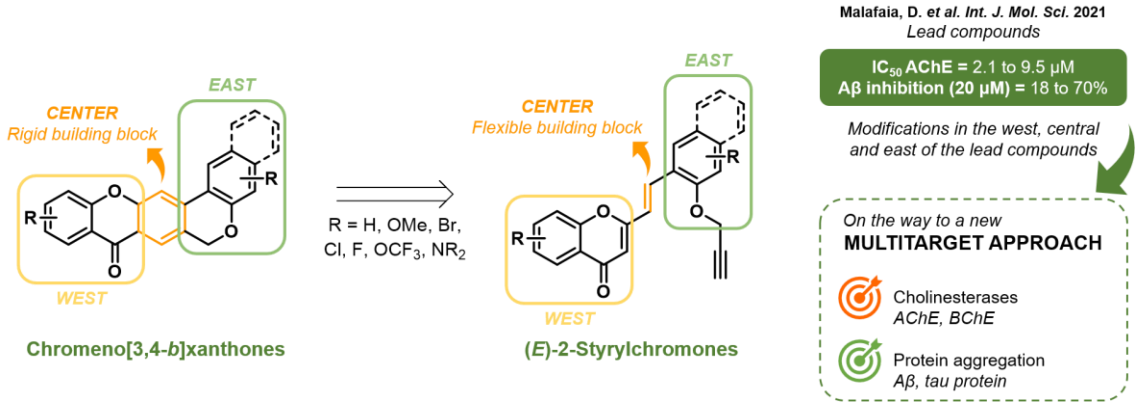

Figure 1. Profiling the multifunctional chromeno[3,4-*b*]xanthones on the way to a new AD multitarget approach.

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Journey to the Chemical Frontier of Urease Inhibition: molecular design guided by machine learning

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Urease is a metalloenzyme that catalyzes the urea hydrolysis and blocking its activity has an important role in both agriculture and medicine as it is essential for *H. pylori survival*. A wealth of molecules has been tested against ureases of different species, but the translation of these molecules still faces limitations related to potency, chemical and metabolic stability, selectivity problems and side effects. It is therefore of interest to screen new compounds and the design of novel compounds would greatly benefit from the insights provided by published scaffolds [1]. To cater to this, we collected and curated the largest set of more than 3200 diverse small molecules from publicly available urease inhibitors to carry a systematic and comprehensive cheminformatics analysis of the activity landscape. We highlighted the different substructures and functional groups for distinct activity and inactivity and meaningful patterns associated with activity. Furthermore, we developed a precise and interpretable urease inhibition classifier using a Random Forest method with high performance. This model was trained using the physicochemical features of compounds paired with docking and protein-ligand fingerprint analysis. We observed a correlation between docking score and compound activity, and the different interactions between ligands and urease were predictors of activity. The best performing model achieved robust accuracy across the training set, and test set. Using a reliability-density neighborhood [2] technique we were able to predict new compounds to be as active as thiourea with 100% precision in the external validation set. Finally, the model was employed on an *in-house* library to identify new leads that were then tested against urease and found to be active *in vitro*. Overall, the results highlight the viability of using machine learning to predict novel urease inhibitors supported by an interpretable machine learning model.

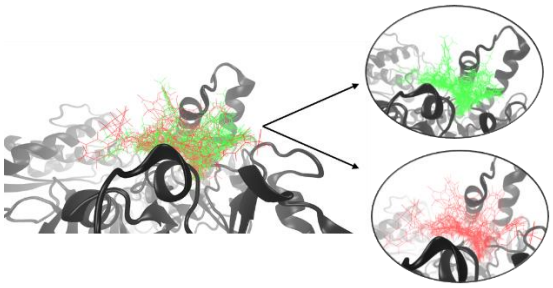

Figure 1. Differences in docking poses between active (green) and inactive (red) molecules.

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Assessment of safety and photo-protective capacity of three selected flavonoids

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Every day, human beings are subjected to environmental stress factors that contribute to premature aging and the development of diseases, with the skin being the main barrier against these factors. Skin is exposed to various external stressors (e.g. ultraviolet radiation (UVR), pollution, smoking), with UVR from the sun as the main cause of several cellular injuries, such as oxidative damage, inflammation, photo-aging, cell cycle dysregulation, and consequently, cancer. As products of the secondary metabolism of plants natural compounds, such as flavonoids, have beneficial bioactivities for humans, such as anti-inflammatory, antioxidant, and anticancer activities, but some of them also show a high photo-protective power. Kaempferol, quercetin, and myricetin belong to the flavonol class, but they have structural differences, namely different hydroxylation, that may lead to differences in their bioactivities. The objective of this study was to understand the influence of structural differences on photo-protective capacity and safety/toxicity of these compounds, using *in vitro* methods.

Cellular safety/toxicity was evaluated at different concentrations and exposure times of the three selected flavonols (kaempferol, quercetin, and myricetin) on HaCaT cells, a skin cell line (human immortalized keratinocytes) using a cell viability assay with the Alamar Blue indicator. Photo-protective capacity was evaluated using sun protection factor (SPF) method, allowing us to understand which is the best photoprotector, and at non-cytotoxic concentrations, allowing us to understand photoprotection at a safe compound's concentration.

HaCaT cell viability was significantly affected by kaempferol and quercetin after 24 and 48 h of exposure, being the effect of myricetin only observed after 48h of exposure. Significant differences were also observed between compounds at the same concentration, indicating different cellular bioactivities. Concerning SPF evaluation, each compound showed a different photo-protective capacity, but all the three compounds showed a high capacity for UVR absorption, indicating high photo-protective capacity. In conclusion, hydroxylation of these flavonols alters their safety/toxicity profile and UVR absorption capacity, resulting in different bioactivities. In addition, exposure time and dose are factors that contributing to the bioactivity of these compounds. This class of flavonoids has a high photo-protective potential.

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Shotgun Proteomics: A Powerful Tool for Investigating the Chemical Complexity of Biscuit Melanoidins

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Maillard reaction (MR) is a non-enzymatic browning reaction that occurs between the carbonyl group of reducing sugars and the amine group of amino acids and proteins, during the thermal processing of various foods. Melanoidins are the final products of this reaction and they are responsible for the sensory properties of foods [1,2]. It has been estimated that melanoidins have a significant presence in the European diet, especially in bread and coffee. Biscuits are also widely consumed, especially by the younger population. Thus, the study of the chemical structure and biological activities of those compounds is extremely important to understand the impact of these foods on consumers' health [2]. Although the exact chemical composition of melanoidins is still unknown, it is believed that melanoidins from wheat-based products are formed from the cross-linking of colored Maillard reaction products (MRPs) with gluten proteins, while other low molecular weight (LMW) MRPs are entangled in the gluten network [2,3].

In this work, a new method was developed to extract melanoidins from biscuits using successive enzymatic digestions and solvents. Proteins involved in melanoidin formation were identified by shotgun proteomics (high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS)). This method was also applied to detect possible protein modifications induced by MR. To validate the effectiveness of this method in that regard, bovine serum albumin (BSA) and gluten model systems were made, reacting each of the proteins with several compounds, such as Glucose, Fructose, Glioxal, Methylglyoxal, Furfural and Glycoaldehyde.

Through the peptide analysis of the melanoidin fraction, it was observed the incorporation of gluten proteins, gliadins and glutenins, as well as peptides from soluble proteins, derived from the wheat flour. Regarding the model systems, it was identified some of the protein modifications, possibly induced by the compounds used in the reactions, showing that this approach is a promising tool to understand the chemical composition and formation mechanism of biscuit melanoidins.

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Insect Flours Potential for Healthy Food

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Worldwide population growth and reducing the global average annual individual carbon footprint are current affairs. Both, posing enormous challenges to all sectors involved in food production with the need to seek new, unconventional source of protein. *InsectERA* PRR Project aims to enable the industrialization and commercialization of innovative products based on insects, both in the food area (animal and human food), as well as in other industries (cosmetics or bioplastics) or in the innovative sector of bioremediation [1]. With the aim to characterize the protein value composition of insect flours from different extraction processes we have developed a sensitive analytical methodology for identification and quantification of Amino Acids. Sample preparation is one of the most variable and time-consuming steps in the analysis of proteins by mass spectrometry, and the quality and reproducibility of sample extraction and preparation significantly impact the results. Strategies have been developed to reduce sample complexity and improve detection. Thus, first was necessary to optimize sample preparation for analysis by extraction and clean-up by solid phase extraction for the analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The method is suitable for the purpose, being a useful tool for the protein value assessment according to the Food and Agriculture Organization of the United Nations tables [2].

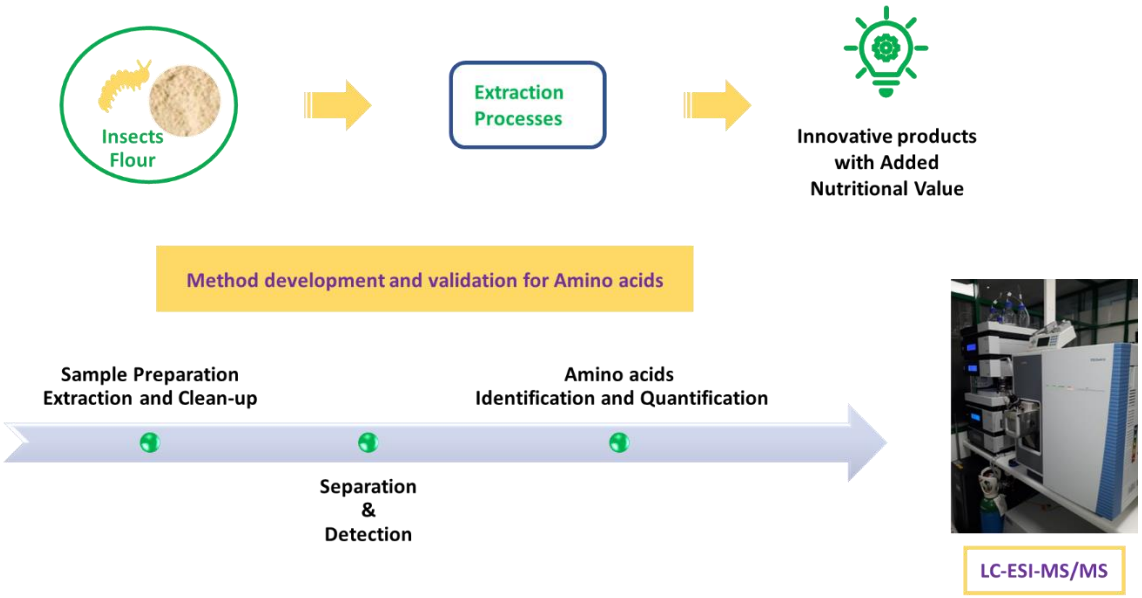

Figure 1. Scheme of method development and validation.

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Development of a dietary supplement designed to prepare a functional food for patients with neurodegenerative diseases

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Neurodegenerative diseases and their progress have been associated to oxidative stress and inflammation.[1] Dietary supplementation with bioactive compounds and essential nutrients such as polyunsaturated fatty acids, vitamin E and minerals may exert both anti-inflammatory and antioxidant functions and have the potential to prevent neurodegenerative diseases.[2] Moreover, some dietary supplementation-based strategies have been demonstrated to be effective in patients with neurodegenerative diseases, especially those with mild cognitive impairment.[2] Supplementation based on multinutrient combinations may provide favorable synergistic interactions between components. It is well known that the Mediterranean diet provides a combination of nutrients that may proffer protection against cognitive decline and a positive effect on neurodegenerative diseases prevention.[3] Thus, we hypothesized that the use of local Mediterranean food products might be used to design new dietary supplements since they are rich in nutrients and bioactive compounds that would work synergistically to counteract many of the pathways that contribute to neurogenerative diseases.
In this work a new dietary food supplement with potential beneficial effects on the condition of patients with neurodegenerative diseases was prepared using some Mediterranean food products produced in the central region of Portugal. Hence, a supplement was formulated to contain white beans, albumin, pine nuts, and grape skins. The composition of this new supplement was analyzed regarding the moisture, ash, protein, and total fat, according to stablished methods (AOAC,2000), and the total fiber (AOAC,1975). In addition, its lipidic profile was also examined by gas chromatography and the *in vitro* antioxidant capacity of the supplement was evaluated using the $ABTS^{\bullet+}$ (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation) scavenging activity method.[4] The supplement showed a content of total fiber of 15.4 ± 0.96 g/100 g and total protein of 24.4 ± 1.35 g/100 g allowing a possible designation of a high fiber and protein food product, according to EU labelling (> 6 g fiber/100 g and > 24 g protein/100 g). The total fat content was 12.04 ± 0.14 g/100 g, of which 1.63 ± 0.36 g/100 g were saturated fatty acids, 4.30 ± 0.73 g/100 g were monounsaturated fatty acids, and 5.61 ± 0.90 g/100 g were polyunsaturated fatty acids. The ratio between polyunsaturated fatty acids and saturated fatty acids showed a good nutritional quality (3.44 ≥ 2). The supplement also showed an *in vitro* antioxidant activity against $ABTS^{\bullet+}$ of 13.2 μmol Trolox equivalents/g of supplement. The results showed that was possible to prepare a nutritive food supplement rich in protein, fiber, and polyunsaturated fatty acids with antioxidant properties that can ensure an adequate nutrition directed to patients with neurodegenerative pathologies.

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Evaluation of nutritional and chemical properties of almonds (*Prunus dulcis*) produced in northeastern of Portugal under different conditions by intercropping systems

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One of the main concerns of agriculture is to provide safe and nutritious food to attend the dietary needs of consumers and to embrace more sustainable production systems, increasing the efficiency of the use of available resources [1]. In this sense, crop diversification in almond orchards aims to improve the quality of the agroecosystem, preventing erosion through sustainable management, as well as mitigating climate change and improving soil quality [2,3]. The objective of this research was to evaluate the nutritional and chemical properties of the almonds, to verify the benefits generated by the interaction between the crops under different cultivation conditions (dry and irrigated soil). The centesimal composition of the samples (protein, fat, carbohydrates, ash, and humidity) was determined through the Official Methodologies of Food Analysis (AOAC), being also calculated the total energy value. The chemical composition was obtained through the quantification of the fatty acid content by GC-FID, the organic acids by a UFLC-DAD system and the sugar content by HPLC-RI. The results showed that, for almonds grown in irrigated soil, they presented a slightly higher fiber, carbohydrate and energy content when compared to almonds from non-irrigated soil. However, for the samples collected from the dry almond grove, they showed a higher content in proteins. For ash, moisture and fat content, no significant changes were shown, revealing that some nutritional aspects were not affected by the different types of cultivation. For the organic acids, the samples collected from the dry almond grove showed higher content of oxalic and malic acid, which can be explained by the stress conditions to which the plant was subjected. Regarding the sugar and fatty acids content, no significant difference was detected between the two samples. Therefore, this research can conclude that the intercropping systems can bring benefits not only to the environment, making production more sustainable, but also maintaining the quality of the almonds under drought stress conditions. Furthermore, future research, such as the one presented, provides support for the development of intercropping systems in water-deficient lands.

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Degradation of vinegar: influence of chemical and microbiological parameters on the quality of wine vinegar

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The word vinegar derives from the French "*vinaigre*". It is a popular food preservative, pickling agent, food ingredient, or beverage due to its antimicrobial and acidulant effects that preserve and increase the shelf life of food products [1,2]. Despite wine vinegar having a long shelf life, its quality decreases with exposure to air, storage conditions, and the presence of microorganisms [1,3]. The objective of this study focuses on the chemical and microbiological evaluation of 22 samples of wine vinegars opened in the first quarter of 2022 to assess their quality and storage life. For this purpose, the presence of ethanol and acetic acid in the samples was quantified by enzymatic kits, the phenolic composition by the Folin method, and the antioxidant activity by the ABTS test. The color was measured using a spectrophotometer at wavelengths 420, 520, and 620 nm. Microbiology analysis occurred through the direct observation of each sample under a microscope, culturing athwart liquid medium, followed by growing in Petri plates. Compared with a recent study, the results revealed an increase in pH, total phenol content, residual ethanol, and decreased antioxidant activity and acidity. Beyond this, the presence of acetic acid bacteria and yeast was noted in some samples. The color parameter demonstrated the darkening of the vinegars and visually some samples started to have sediments. Overall, this study highlights the quality, freshness, shelf life, and importance of chemical and microbiological screening of wine vinegars against storage conditions.

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Protein extraction from *Arthrospira platensis* for use in food processing

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Algae protein has emerged as a sustainable and non-allergenic alternative to animal protein for use in the food industry as the market seeks to reduce reliance on traditional animal protein sources and promote more sustainable and health-conscious food options. The development of an efficient protein extraction and quantification method with an easily scalable approach is crucial for the use of protein isolates from *Arthrospira platensis* in various applications such as improving the nutritional value of animal feed, healthier foods, and nutraceuticals. Therefore, different extraction methods were investigated to improve the extraction of high purity protein from the algae. The total and free amino acid profiles of the protein were determined by HPLC-DAD, while SDS-PAGE was used for protein characterization. This study shows the potential of *Arthrospira platensis* as a sustainable and non-allergenic protein source for food production and, proposes a simple and economical approach to algal protein extraction that could be used in large-scale production. However, further research is needed to assess the commercial viability of algae-based protein and to overcome challenges related to taste, texture, and consumer acceptance.

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Electrochemical polymerization of catecholamines and catechol derivatives for optical and electrochemical biosensors

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When developing biosensor interfaces, there is a need to create efficient functional coatings that are able to robustly attach biologically active recognition elements (such as enzymes or antibodies), allowing proper transduction signals, namely electrochemical or optical. Thus, searching for sustainable materials and straightforward surface chemistries is still a prevalent research topic in this field. Catechol derivatives have inspired the development of a wide range of functional materials mimicking the natural adhesives of marine organisms [1], but are still not greatly employed in biosensing interfaces. Oxygen-driven polymerization of catecholamines in mild basic aerated solutions is the easiest and standard method to prepare biomimetic polymeric films onto a broad range of surfaces. However, electrochemical routes emerged in recent years [2-4], as efficient synthetic alternatives to overcome problems of reproducibility and chemical heterogeneity, known to occur during chemical synthesis. Polydopamine (PDA) is by far the most investigated catechol-derived coating, though, there are other promising catecholamines possessing additional chemical groups [5], whose synthesis and physicochemical properties are not yet fully discussed in the literature.
This work aims to explore the electrosynthesis of highly adhesive and biomimetic polymeric interfaces, namely polydopamine, polynorepinephrine, polydopa, polycatechol and other derivatives, suitable for biosensing platforms. An accurate control over the growth charge, mass and thickness allows the synthesis of adhesive polymer films with different chemical composition, redox properties, ionic permeability and wettability. To overcome the poor conductivity of the polymeric coatings, we have optimized the electro-co-polymerization of catecholamines and pyrrole, to combine the catechol groups, suitable for functionalization with biomolecules, with the high conductivity of polypyrrole, useful for electron transfer events. The performance of the polymeric matrices was evaluated in amperometric biosensors for the detection of phenolic compounds, using the enzyme laccase, and also in protein affinity sensors, by monitoring the specific antibody/antigen interaction with real-time surface plasmon resonance assays.

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An electrochemical sensor based on transition metals for detection of bioactive molecules containing phenolic groups

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Nowadays, chemically modified electrodes are increasingly attracting more and more interest in the field of electrochemical analysis. The electrochemical signals, as well as analytical performance of the electroanalytical methods, can be significantly improved through the modification of the electrode's surface with nanostructured materials and/ or with materials with electrocatalytic activity. Transition metal-based materials (TMs, such as Mn, Fe, Co and Ni) are a typical class of non-precious metal-based materials that show facile redox properties and exhibit good catalytic performance in different applications, such as supercapacitors, batteries and sensors [1]. Bimetallic TMs has been adopted to modulate and optimize the electronic configuration of different atoms to yield better catalytic effects. It has been proven that bimetal TMs-based materials perform better than the monometallic counterpart [1].

Carbon-based nanomaterials such as carbon nanotubes (CNTs) and reduced graphene oxide (rGO), have been used to anchor TMs to improve the stability by preventing the dissolution and/or agglomeration of the materials deposited at the electrode surface [2,3]. In addition, the electrical conductivity can be improved and the electronic state of TMs modulated in the presence of carbonaceous supports, due to the strong metal-support interactions that may improve the electron transfer kinetics at the metal/interface [4].

In this communication, we demonstrate the use of bimetallic CoNi-based materials supported on different conductive carbon materials, including rGO and carbon xerogel (CX) to enhance the sensitive detection of bioactive molecules containing phenolic groups. Hydroquinone (HQ) was used a model molecule, and our approach was proved successfully in the detection of bisphenol A (BPA) and dopamine (DA). The results show that the introduction of CoNi metals on the carbon structure can significantly improve the electrochemical response toward the different phenolic compounds detection under analysis. The electrochemical activity of CoNiP@rGO and $CoNi_R$@CX exhibited a good sensitivity for HQ detection of 1.81 A M^{-1} cm^{-2} and 3.32 A M^{-1} cm^{-2}, respectively, estimated from cyclic voltammetry. When CoNiP@rGO was studied for BPA detection, a low limit of detection (LOD) of 0.38 nM and a high sensitivity of 96.4 A M^{-1} cm^{-2} were achieved by DPV. Finally, $CoNi_R$@CX was employed for the electrochemical sensing of DA with a sensitivity close to 1.55 A M^{-1} cm^{-2}.

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Simultaneous voltammetric determination of carbamazepine, paracetamol and naproxen using a miniaturized electrochemical device

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The exponential increase in pharmaceuticals' production and consumption is evidently connected with their increased excretion which leads to the contamination of surface and underground waters with drugs and drug residues. Unfortunately, wastewater treatment plants (WWTP) are not designed to degrade these highly specific compounds. Therefore, the monitoring of pharmaceutical concentrations in environmental matrices, mainly aquatic, is essential. Although the reported concentrations are generally low (usually less than 1 μg L^{-1}), the authorities are concerned about the long-term impact on living beings [1].

To address this problematic critical issue, conventional electrochemical methods have been proposed. However, despite being very attractive, there is a need for portable handheld devices suitable for on-site applications and capable of data acquisition without specialized software and external devices [2].

It is also very important to study mixtures of several pharmaceuticals since they can coexist in water courses. We have previously evaluated the possibility of qualitative separation between carbamazepine (CBZ), paracetamol (PAR) and naproxen (NPX) [3].

The objectives of this work have been (1) to test the miniaturized potentiostat device with potassium ferricyanide as redox probe compound, (2) to analyse the mixtures referred above with the developed device and finally (3) to compare the data acquired with a commercial potentiostat (PGSTAT302N, Metrohm) with the output of our developed solution.

The techniques performed have been cyclic and differential pulse voltammetry and chronoamperometry. The analysis of the results will allow to evaluate and to improve the new device's performance to ultimately test it on river water samples.

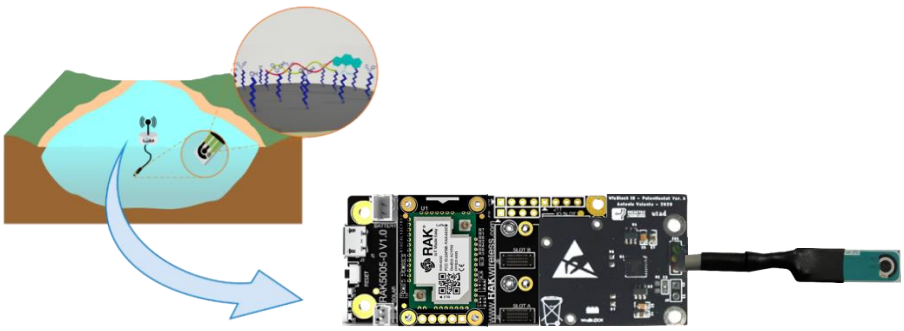

Figure 1. Scheme of the miniaturized electrochemical device and its application.

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Valorizing electrochemical multiplexing of humoral immune response biomarkers for precision chronic diseases medicine

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Chronic diseases such as Systemic Lupus Erythematosus (SLE), Sjögren's syndrome (SS) and Alzheimer disease (AD), which are considered long-term conditions with persistent and degenerative effects and no cure, are becoming increasingly common and a priority for action in the healthcare sector. They have a significant impact on people's quality of life and have important social and economic consequences, so it is important to detect them as accurately and early as possible. The significant advantages demonstrated by autoantibodies (Abs) as biomarkers for such a purpose have prompted the search for new specific autoantigens to aid in the minimally invasive diagnosis and monitoring of prevalent chronic diseases. In this context, and with the aim to identify particular autoantibody (Abs) signatures and demonstrate their diagnostic potential, our research group has recently developed disposable platforms for the rapid, simple and reliable electrochemical multidetermination of the individual and total content of the most relevant immunoglobulin (Ig) classes (IgG, IgM and IgA) of Abs against dsDNA (dsDNA-Abs) [1], and Abs against four extractable nuclear antigens (ENA-Abs): anti-La/SSB, anti-Ro/SSA, anti-RNP70 and anti-smRNP [2].

These bioplatforms use as a base magnetic microparticle beads (MBs) modified with a biotinylated human dsDNA prepared in the laboratory or the specific nuclear antigen for the efficient capture of the corresponding Abs. Captured Abs were enzymatically conjugated to HRP-labeled secondary antibodies and the resultant magnetic bioconjugates were trapped on the working electrodes of quadruple disposable platforms to perform amperometric transduction. The variation of the cathodic currents recorded at a potential of -0.20 V (vs an Ag reference pseudoelectrode) in the presence of hydroquinone (HQ) as redox mediator and H_2O_2 as enzyme substrate was directly proportional to the concentration of the target Abs.

Both electroanalytical biotools demonstrated excellent analytical and operational characteristics and the potential to discriminate healthy subjects easily and reliably from patients diagnosed with SLE, SS, and AD. Their unique features make them a very attractive alternative to conventional ELISA methodologies in terms of simplicity, cost, time test, and point-of-care applicability for the detection and follow-up with improved precision (due to the multiplexed nature of the determination) of these degenerative diseases.

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Genetic biosensor for fast and selective SARS-CoV-2 detection

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The recent pandemic has highlighted the importance of developing fast, innovative and reliable biosensing solutions that can be applicable *in situ* using a point-of-care approach [1]. One of the initial problems that helped the virus fast progression was the lack of sensitive tests, thus we relied only on temperature screenings and then of rapid-antigen tests. But both present problems, since not all the infected individuals developed fever and the rapid-antigen tests had a very low accuracy percentage. Moreover, they were most effective at the peak of infection, which further boosted the infection rates.
Here we report a novel nanotechnology genetic biosensor for SARS-CoV-2 identification that relies on the most efficient nature process: hybridization due to complementarity. The sensing process takes 5 minutes, it does not require amplification or purification steps and it can be applied *in situ* by non-specialized personnel. We successfully targeted regions in the ORF1ab, E and N genes and performed an IVD European Union certification. This technology was patented and licensed to the industry.
The developed biosensor is an easily adaptable device that allows the recognition of new SARS-CoV-2 variants by changing or adding a new probe. This is a major advantage due to the virus high mutation rate, which is one of the emerging challenges regarding the current pandemic [2].

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Engineered functional photonic materials for bioinspired sensing

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Biosensors are constantly evolving to develop more sustainable solutions that can be used cost-effectively for diagnostic purposes. Given the challenge of reducing premature deaths from non-communicable diseases, the control and management of these diseases is of paramount importance. A critical issue is the need to detect early stages of disease. This is driving scientific progress in biosensors, as most laboratory-based methods are lengthy, expensive, and not sensitive enough.
Among the label-free approaches in optical biosensing, the use of structural colors from photonic crystals stands out due to its non-fading features and easy integration with soft biomaterials, which can be engineered to be stimuli-responsive. Structural colors in nature arise from physical mechanisms of light interaction with matter, resulting in the most amazing colors, such as the iridescent colors in feathers of birds or the camouflage ability of chameleons. Several works presented here show the synthesis of different bioinspired photonic nanostructures and novel hybrid materials that are used as optical transducers in the development of biosensors.
To further explore bioinspired and biomimetic concepts, molecular imprinting technology has been applied to develop imprinted polymers and hydrogels that mimic natural molecular recognition and have been successfully tailored to detect disease biomarkers, such as circulating proteins and extracellular vesicles. These imprinted materials enable selective recognition and can be nanostructured to form a sensing layer that reflects a specific color. The responsiveness of the soft photonic materials in the presence of the target analyte leads to optical changes that are used for detection and quantification, making them useful label-free sensors. In particular, the use of natural biopolymers (e.g., proteins, carbohydrates) in the design of these biosensors is a novel and sustainable alternative, for which several examples are given.
Advances in biosensors that integrate functional photonic materials are expected to enable sensitive and selective detection of biomarkers. In the future, they could compete with standard diagnostic methods and provide healthcare systems with cost-effective tools for real-time disease detection and monitoring.

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Biomimetic hydrogel for optical sensing of extracellular vesicles

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Nano-sized extracellular vesicles (EVs) are essential mediators of cell-cell communication, as they carry information, in the form of genetic material, proteins, and lipids, from the parent cell to another cell. Upon reaching the receiving cell, they release their content, triggering a change in its behavior [1]. When this transport system is disrupted, this lack of control naturally contributes to many diseases, including cancer, neurological and cardiovascular diseases [2-4]. Biosensors can assist in retrieving small quantities of EVs from a complex mixture of circulating substances. At the same time, they can also detect and/or quantify EVs, which is not always possible with current standard methods. In this way, they would act as highly valuable diagnostic methodologies in assessing clinical stages of diseases associated with EVs. This work aims to develop synthetic imprinted polymers in combination with an optical detection method. The molecular recognition sites, consisting of a set of selective complementary interactions, were produced in a cross-linked polymer matrix, and obtained after removal of the target template used. For optical detection, photonic crystals have shown successful applications in the detection of various disease biomarkers [5]. The final product is a photonic hydrogel with a specific structural color that changes its optical properties upon detection of the intended target. In the near future, the aim is to have innovative optical biosensors capable of detecting and identifying small amounts of EVs in circulating fluids at low cost and with high accessibility and applicability.

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NO2Probe: application of a new nitrite point-of-care test in Biomedicine

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Periodontal Disease (PD) is a chronic pathology with high prevalence in the community, affecting 80% of the world's adult population [1]. This inflammatory condition involves the immune response to bacterial biofilms that result from the lack of oral hygiene, malnutrition, and some medications. The inflammatory process affects tissues around teeth (bone and gingival gums), which in the worst-case scenario, can lead to tooth loss [1],[2]. Although PD has no cure, timely treatments can retard its progression. Currently, the diagnosis of PD is made through the assessment of gingival tissue morphology (periodontal charts) complemented with RX radiography, a long procedure executed by clinicians during medical appointments [3]. For the early diagnosis of PD and the easy monitoring of the disease's progression and treatment, quick and simple-to-use methods to measure specific (bio)markers are required. Some clinical studies have shown that the levels of nitrite (NO_2) in saliva increase with PD's progression, so this ion has been suggested as a potential biomarker [4]. However, none of the current methods for nitrite quantification enable the quick and reliable analysis in such complex samples [3]. Our group has developed a novel point-of-care test (POCT) for nitrite analysis - the NO2Probe, which consists of a miniaturized electrochemical biosensor composed of a carbon screen-printed electrode modified with cytochrome c-type nitrite reductase (ccNiR) as the biorecognition element. The electrodes were also modified with a new mono-enzymatic scavenger system to deplete molecular oxygen (O_2) from saliva samples [5]. This way, the electrocatalytic currents evolved in the presence of nitrite can be detected under ambient air, either using voltammetric or chrono-amperometric techniques. Herein, we tested and validated the NO2Probe in saliva samples through a pilot study conducted in the Egas Moniz Dental Clinic. A total of 38 participants with (N=27) or without (N=11) PD were enrolled in the study and provided a saliva sample that was analyzed on-site by the NO2Probe. The analyses were repeated after the samples' centrifugation and/or freezing. All results were compared with those delivered by the gold standard method (Griess reaction). The statistical analysis (t-student test) of nitrite concentrations obtained by the two techniques showed that the values are comparable ($p<0.05$), thus validating the NO2Probe. Our results also demonstrate that the use of NO2Probe does not require the centrifugation of saliva samples, since the degree of turbidity does not affect the results. Another important outcome is that saliva freezing without previous centrifugation should be avoided since the nitrite concentration goes up, probably due to the nitrate-reducing bacteria present in saliva [6]. On the contrary, in frozen saliva samples, the nitrite levels decrease, probably due to its instability. Therefore, saliva should be analyzed while fresh, at the point of care.

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Extracellular vesicles increased production by stimulation with nitric oxide releasing polyurea biodendrimers

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Extracellular vesicles (EVs) are phospholipid bilayer membrane vesicles secreted to the extracellular environment by almost all type of cells. Rich in multiple cargoes, including nucleic acids, proteins and lipids, EVs were initially described as cellular waste, but later findings suggest a more complex role. In fact, EVs are biomolecules carriers and key players not only in cellular communication processes, helping the homeostasis maintenance, but also in diseases progression. Due to their role, EVs have emerged as a new cell-free strategy with clinical applications that may include disease diagnosis, prognosis and therapy, working as non-invasive biomarkers and drug delivery vehicles, with high effectiveness and sensibility [1]. Despite the recognized potential, a successful clinical implementation is still limited by some technical challenges, mainly on reliable and effective production platforms capable of delivering large EVs amounts, while maintaining quality and bioactivity. In this sense, multiple studies have been conducted focused on the development of complementary strategies that stimulate EVs secretion and improve the production yield. These include approaches that extend from modulation of cell culture conditions and parameters, through the addition of chemical, physical or environmental stresses [2]. In this work, we propose cell culture supplementation with polyurea oxide (PURO) biodendrimers, in order to stimulate EVs production. These soft nanoparticles are non-toxic, and are reported as modulators of cell behavior, triggering osteogenic differentiation of human mesenchymal stem/stromal cells [3]. Importantly, our results show that PURO supplementation increases intracellular levels of nitric oxide, being intimately linked to an increase in EVs production (2.5-fold) (Figure 1), without causing a significative increase in cell toxicity.

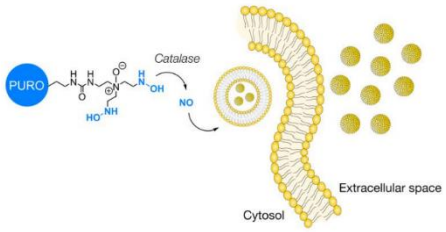

Figure 1. Mechanism of extracellular vesicles secretion triggered by a PURO biodendrimer.

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Exploring the therapeutic space around mitochondrial respiratory chain to tackle chronic diseases

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Mitochondrion, an intracellular organelle with a double membrane and distinctive soft architecture, is a hub of eukaryotic cell functionality that ensure the physiological requirements of multicellular life. So, it is not strange that mitochondrial dysfunction can be connected to almost all chronic diseases, including those that affect the systemic metabolism (e.g., type 2 diabetes) and the brain networks connectivity (e.g., Alzheimer´s and Parkinson´s diseases, AD and PD) [1]. Although several faces of mitochondrial dysfunction can be revealed by different diseases, a deep analysis reveals that all pathological roads start or converge to the inner mitochondrial membrane with a dysfunctional mitochondrial respiratory chain. Thus, we are exploring the pharmacological space around mitochondrial respiratory chain to develop an innovative a therapeutic tool to resolve the puzzle of many human chronic diseases. The therapeutic tool is a lipid-based nano-system, named SC-Nanophytosomes, built with elderberry anthocyanins (*Sambucus nigra* L.) and membrane polar lipids from *Codium tomentosum* (a green algae). Elderberry anthocyanins were selected by their ability to overcome the anomalous activity of the mitochondrial respiratory chain (mainly at level of complex I), working as membrane electron carriers that oxidize NADH and deliver electrons for complex III [2]. Algae membrane polar lipids, rich in the anionic phospholipids (mainly in cardiolipin precursor) and high n-3/n-6 PUFA ratio, were selected to modulate the lipid composition of the inner-membrane membrane and by their self-assembling properties in lipid vesicles with competence entrap and preserve the anthocyanins in the flavylium cation form [3]. Using a rotenone-induced PD rat model, it was shown that SC-Nanophytosomes, delivered by drinking water for three weeks, have competence to: i) mitigate PD-related motor disabilities, ii) decrease the brain levels of α-synuclein, iii) mitigate, the impaired mitochondrial respiratory chain functionality in brain and skeletal muscles tissues, iv) counteract the PD-related changes on the fatty acid profile of mitochondrial lipidome; v) modulate cell redox state and the activity of antioxidant enzymes [4]. Therefore, SC-Nanophytosomes support a new concept of mitochondria-targeted therapy for neurodegenerative diseases with application for many other chronic diseases.

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Efficient models for Monte Carlo simulations applied to different biomolecules-based drug delivery systems

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Our group has, for some time, been developing successful models for the description of the adsorption behavior of charged polymers in complex systems governed by electrostatic interactions [1,2]. These are known to impact on diverse aspects of biomolecule-based-systems, namely those directed at drug delivery. Examples include the use of nucleic acids, polymers and peptides to design different types of nanotransporters to improve drug delivery, for a broad range of diseases that include cancer. In some cases, the overall behavior is dictated by electrostatic interactions, in others electrostatics play an indirect, although critical role.

In this work, we propose to explore this topic resorting to Monte Carlo simulations and simple coarse-grained models. The idea is to reduce the inherent complexity of the systems focusing on a small set of parameters, such as overall chain charge, length and charge distribution, in order to assess their impact on the overall behavior of the systems.

As case studies we have focused on the use of DNA nanostructures and/or nanoparticles modified with polymers such as hyaluronic acid or with cell penetrating peptides, that have been recognized as promising approaches to increase the efficacy of chemotherapeutic agents, improve targeting, enhance biological barrier crossability and specific cellular uptake, thus reducing side effects and circumventing resistance of tumors [3-6].

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Addressing hard-to-access brain tumors using a chemo-photothermal nanotechnology

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Introduction: Glioblastoma (GB) is the most common and lethal form of primary brain tumors, characterized by marked heterogeneity and adaptability leading to chemo- and radioresistance to conventional therapies and recurrence. The lack of specificity to the tumor tissue and the existence of the dual blood-brain/tumor barrier lay down as additional obstacles. Up to now, there is no definite solution to GB.

Hybrid gold-lipid nanoparticles (HNPs) are herein hypothesized to tackle these issues. Novelty stems from the design of HNPs constructed by covalent binding between organic (ultra-small nanostructured lipid carriers, usNLCs) and inorganic nanoparticles (gold nanorods, AuNRs, with photothermal therapy capability), using c(RGDfK) that serves the dual purpose of the linker and tumor-targeting peptide. The HNPs were further functionalized with transferrin ($HNPs^{Tf}$) as a blood-brain barrier ligand. Thus, this strategy combines a chemical attack owing to celecoxib as repurposed drug incorporated into the solid-matrix lipid nanoparticle and a physical attack through hyperthermia assigned to gold nanorod moiety, which along with the dual targeting approach may dramatically boost the possibilities of therapeutic success.

Methods: usNLCs, obtained from the hot high-pressure homogenization technique, and AuNRs, prepared from the seedless method, were covalently bound via amine carbodiimide coupling, and structurally analysed through nuclear magnetic resonance spectroscopy. The HNPs obtained were assessed in terms of physicochemical characteristics, including particle size, zeta potential, polydispersity, drug loading, and photothermal properties. The *in vitro* performance of the HNPs regarding permeability through HBMEC, cytotoxicity and cellular uptake efficiency using HBMEC and U87 cells, and cell apoptosis of U87 cells was evaluated. Finally, the *in vitro* findings were further confirmed by *in vivo* studies, such as biodistribution, efficacy studies in an orthotopic glioblastoma mouse model through magnetic resonance imaging (MRI), and toxicity evaluation.

Results: The HNPs presented a particle size below 100 nm, a low polydispersity index (ca. 0.200), high drug loading (5% w/w), and photothermal behavior dependent on the AuNRs concentration. The *in vitro* and *in vivo* studies revealed that $HNPs^{Tf}$ could safely and specifically increase the permeability of the blood-brain barrier via receptor transferrin and facilitate the accumulation of nanoparticles in the tumor region in orthotopic tumor-bearing mice. Furthermore, chemo- and photothermal therapy enhanced the therapeutic effect in glioblastoma, inhibiting the tumor volume growth by 71%. A 113% delay in tumor growth was also observed in mice administered the irradiated HNPs compared to 43% in the control saline. This resulted in prolonged survival of the tumor-bearing mice along with a favorable side effect profile.

Conclusion: In conclusion, hybrid gold-lipid nanoparticles represent a multipurpose strategy that integrates desired chemo-/photothermal synergistic therapeutic functions and improves brain drug delivery and anti-glioma treatment efficacy.

To evaluate the potential synergistic effects of chemo/photothermal functionalities of HNPs against glioblastoma.

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Oral films incorporating chestnut shells bioactive compounds as delivery system for the prevention/treatment of oral mucositis

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Oral mucositis (OM) is one of the most common side effects of antineoplastic treatments, being characterized by an acute inflammation caused by oxidative stress. The OM symptoms include pain, bleeding, ulcers, with a consequent difficulty in speaking and swallowing, and secondary infections, all of which can compromise the treatment of the primary disease and its outcome [1]. Oral films are alternatives to conventional pharmaceutical dosage forms that can act as delivery systems, disintegrating rapidly, releasing the active molecules, improving the oral absorption with local effects, and being easily placed in the oral cavity. Chestnut (*Castanea sativa*) shells are an agro-industrial by-product with well reported bioactive properties, including antioxidant, anti-inflammatory, anticancer and antimicrobial activities [1]. On our last study we optimized a chestnut shells extract by Subcritical Water Extraction (SWE), reporting its potential as active ingredient against OM [2]. In this study we aim to formulate an oral film with SWE *C. sativa* shells extract, evaluate its physicochemical properties, and conduct *in-vitro* and *ex-vivo* studies to screen its potential use in OM condition. More than 15 formulations were tested using different polymers, plasticizers, and concentrations trhough solvent casting method. Methocel 1000 with SWE *C. sativa* shells extract produced a fast-dissolving film, with optimal thickness (0.125 mm), uniformity, tensile strength (11.4 N), extension (37.6 mm), and excellent folding endurance. The results attested that the oral film of SWE *C. sativa* shells extract is a novel and potential alternative to available commercialized products, probably resulting in improved patient adherence and efficiency to the treatment of OM.

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Synthesis of Carbon Dots Using Dendrimers as Co-Precursor

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Carbon dots (CDs) are a relatively new type of carbon nanomaterial receiving great interest from academia and industry. CDs are mainly composed of carbon with sp^2/sp^3 hybridization, along with assorted surface functional groups [1]. The main feature of CDs is photoluminescence (PL), and therefore, the primary focus is the design and synthesis of CDs with controlled and superior PL properties [2]. Several aspects are taken in consideration to reach the desired properties, such as the choice of the synthesis method, solvent, starting precursor, and reaction parameters [2]. In fact, CDs with enhanced PL properties are often obtained using bottom-up approaches (solvothermal or microwaved) and doped/co-doped with heteroatoms such as N or S, but also through surface functionalization with N-type molecules [3]. In this regard, dendrimers which are monodispersed polymeric molecules can serve as precursors for the synthesis of carbon dots with promising results for biomedicine and sensing applications [4,5,6].
In this work, carbon dots are synthesized using N-type dendrimers and ascorbic acid (main precursor) using the hydrothermal method and optimized reaction conditions. After synthesis, the crude material is subjected to centrifugation and dialysis. The purified CDs are characterized using UV-Vis and fluorescence spectroscopies to evaluate the optical properties (absorption, multi-color emission, and quantum yield), and the surface functional groups identified by NMR and FT-IR. The morphology, size, and ζ-potential of the prepared CDs will be evaluated by TEM, AFM, and DLS. The biological applications of the developed dendrimer-based CDs are also assessed through the gene delivery, cytotoxicity, and cellular uptake studies. Furthermore, the first results on the chemical functionalization of CDs with low-generation N-type dendrons will also be presented and discussed.

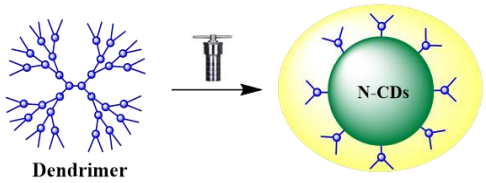

Figure 1. Schematic representation of the dendrimer-based carbon dots.

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Breaking Through the Blood-Brain Barrier: The Synergy of Machine Learning and Molecular Simulations in Designing Cell-Penetrating Peptides for Glioblastoma Nanoparticle Therapy

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Chemotherapy for glioblastoma has provided limited benefit due to the failure of drugs to penetrate the Blood-Brain Barrier (BBB) and non-selective drug accumulation in the entire brain. In this work, different active targeting molecules were evaluated for the usNLCs surface modification, including cell-penetrating peptides (CPPs), tumor-targeting peptides, receptor proteins, and cationic surfactants, in order to improve the transport of the drug across the BBB, and consequently target brain tumor cells. [1-3] We hypothesize that the surface functionalization of ultra-small nanostructured lipid carriers (usNLCs) with a novel class of cell-penetrating peptides (CPPs) could enhance their biological barrier crossability, increase their specific cellular uptake, and ultimately promote the delivery of the nanoparticles to target cells. The usNLCs were functionalized with distinct biomolecules adsorbed on the usNLCs surface and its ability as a targeting approach to BBB (HBMEC) and glioma cells (U87 cells) were evaluated in terms of physicochemical properties, cell uptake, permeability in the 2D-BBB model, and the tumor growth inhibitory ability. Formulations comprised several targeting strategies, including nanoparticles negatively and positively charged, which were further functionalized with CPPs differing in terms of amino acid composition, charge, and molecular weight, and also receptor-mediated molecules such as c[RGDfK], or transferrin.
Machine learning methods [4] were used to predict those CPPs that are most likely to bind to and penetrate glioblastoma cells and those that are likely to have high efficacy in delivering nanoparticles to glioblastoma cells, based on known CPPs and their properties, such as charge, hydrophobicity, and secondary structure. Molecular Dynamics simulations allowed describing the interactions between CPPs and the model cell membrane, as well as between the peptides and the usNLCs. Monte Carlo simulations allowed describing the electrostatic-driven adsorption of the different CPPs to the usNLCs surface, by quantifying the adsorption degree of each model CPP chain, the coverage of the nanoparticle surface and the overall adsorption patterns of CPPs.
The best performance in terms of permeability in the 2D-BBB model was obtained with transferrin, followed by CPP4. However, the cellular internalization was higher for CPP4. BBB cells were more sensitive to the nanoparticles than glioblastoma cells. Functionalized-usNLCs were capable of the transportation of the celecoxib (CXB) into living cells and the cellular-uptake mechanism was activated on more than one route in an energy-dependent or -independent manner. The internalization was 2.5 times higher in glioblastoma cells than in the BBB cells, which could be favorable in the case of brain tumors. This study provided valuable insights into the mechanisms of cellular uptake of nanoparticles and allowed optimizing the design of the nanoparticles to improve their binding and penetration efficiency towards a promising therapeutic strategy for the treatment of GB. The functionalized-usNLCs showed high affinity to BBB cells and tumor cells. The dual-targeting approach of usNLCs could significantly contribute to BBB transport and tumor growth inhibition.

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In vitro and *in vivo* antioxidant properties of *Actinidia arguta* leaves obtained by Ultrasound assisted extraction

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Actinidia arguta fruit (kiwiberry) is a small grape-sized fruit, characterized by a hairless skin and a pleasant flavor, having many benefits for human health [1,2]. During production, leaves are removed to promote a better fruit maturation [3]. The aim of this study was to screen the in vitro and in vivo antioxidant effects of *A. arguta* leaves extract to demonstrate its potential as nutraceutical ingredient. Briefly, dried leaves of *A. arguta* were extracted by UAE using water as solvent and a solid: liquid ratio of 10 % (w/v) during 31.11 min, maintaining an ultrasonic intensity of 30 W/m^2 [3]. After extraction, samples were filtrated, lyophilized, and characterized regarding total phenolic content (TPC) and antioxidant/antiradical capacity (ABTS, DPPH and FRAP assays). Afterwards, cell viability assays were performed in intestinal cell lines, namely Caco-2 and HT29-MTX, in concentrations ranging between 0.1 and 1000 µg/mL. The in vivo assessment of the redox markers and biochemical profile were evaluated in wistar rats (n = 6 / group) after oral administration of *A. arguta* leaves extracts (50 and 75 mg/kg bw) for 7 days, using commercial kits. Water was used as negative control and vitamin C as positive control. The results demonstrated that the extract has a high TPC (97.50 mg of gallic acid equivalents (GAE)/g of dry weight (dw)) and strong antioxidant/antiradical activities (IC50 = 249.46 µg/mL for ABTS assay; IC50 = 547.34 µg/mL for DPPH assay; 1440.13 µmol of ferrous sulfate equivalents (FSE)/g dw for FRAP). The MTT assay revealed that the extract led to a decrease of the HT29-MTX viability to 93.54% after exposure to the highest concentration tested (1000 µg/mL), while the Caco-2 cells viability was not affected.The in vivo studies attested that the administration of *A. arguta* leaves extracts increased the levels of superoxide dismutase (183.36 and 175.26 units/g protein), glutathione peroxidase (205.35 and 133.60 units/g), and catalase (3,435,762 and 7,840,180 nmol/min/g protein) in livers and kidneys,respectively, being significantly different from controls (water and vitamin C). Regarding triglycerides levels, the values were significantly lower in animals treated with *A. arguta* leaves extracts when compared to the control groups. These results highlight the antioxidant potential of *A. arguta* leaves extracts as potential preventive ingredient against oxidative diseases progression. Further studies should be performed to identify the metabolites responsible for these activities.

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Boosting the intrinsic SOD-like activity of carbon nanomaterials

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The investigation of carbon-based nanomaterials as SOD mimics dates back to 1995 when it was observed that fullerenes exhibited superoxide anion radicals ($O_2^{\bullet-}$) scavenging activity [1]. Since then, several carbon nanomaterials have been proposed as SOD substitutes, namely, hydrophilic carbon clusters (HCC) [2], graphene quantum dots (GQDs) [3], and oxidized activated charcoal (OAC) [4]. While fullerenes and HCC require post-synthesis treatments to surpass water-solubility issues, OAC raises some concerns due to their potential toxicity along with fullerenes. On the other side, GQDs are considered biocompatible and non-toxic but their SOD-like activity is usually very low, requiring doping processes or the assembly of a hybrid nanozyme in time-consuming and expensive steps [5]. Herein, we propose a new member of the carbon nanomaterials family to overcome the above-mentioned limitations. The new nanomaterial is produced galvanostatically from graphite using a phosphate buffer as the electrolyte in a one-step, simple, fast, and cost-effective method. The physical-chemical properties assessed by several characterization techniques demonstrated that these nanomaterials exhibit a structure dominated by sp^2 carbons in a non-order carbon network functionalized with carboxylic, hydroxyl, and mainly epoxide groups. The SOD-like activity was evaluated using the hypoxanthine-xanthine oxidase system as $O_2^{\bullet-}$ generator and the reduction of nitro blue tetrazolium chloride (NBT) as the detecting system. The results revealed that the carbon-based nanomaterials exhibit a low SOD-like activity, however, a significant boost in the activity was found by the addition of an electrolyte to the as-prepared nanomaterial solution. The role of the nature of the electrolyte cation (Na^+ and K^+) as well as its counterion is discussed. The effect of the ionic strength of the synthesis solution and its composition on the SOD-like activity of the as-prepared nanomaterial was also analyzed. The results revealed that the intrinsic SOD-like activity increases nearly linearly with the ionic strength of the solution where they were generated. It is hypothesized that the electrostatic interactions promoted by Na^+ or K^+ and PO_4^{2-} drive nanomaterial´s organization for an architecture that allows a strong SOD-like activity. The SOD-like activity of the sodium carbon nanomaterials was also compared to commercially available GQDs as well as to a native SOD enzyme. Based on these studies, it was selected the best synthesis conditions to prepare a SOD-like carbon-based nanomaterial to be tested in cell viability studies. It was shown that carbon nanomaterials with a high ionic strength display no toxicity for human neuronal SH-SY5Y cells, and human keratinocyte HaCaT cells. Hence, this study provides a starting point for the development of a new nanotool to fight the oxidative stress associated with pathophysiological conditions where the SOD activity is depleted.

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Multifunctional SPIONs coated with Dextran or Leaf Extract for Theranostic Application

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Magnetic nanostructures have been attracting huge interest as a rapidly growing class of magnetic materials for many applications, especially for biomedical applications in cancer treatment, since they present unique properties that make them suitable for magnetic hyperthermia treatment (MHT) and resonance imaging (MRI). Moreover, they can be further functionalized with gold and gadolinium and be also suitable for T1/T2 weighted images [1,2].

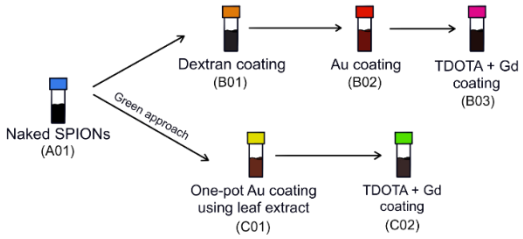

Figure 1: *Schematic drawing of the two functionalization routes, one using dextran and the other being the leaf-extract-based (LV) one-pot functionalization.*

In this work we present the effect of the functionalization of nanostructures based on core–shell magnetic iron oxides (Fe3O4) displaying superparamagnetic behavior (SPIONs). Iron oxide nanoparticles were synthesized by co-precipitation with controlled pH process following the method given by Saraiva et al. [3]. SPIONS were coated with dextran followed by a gold coating and functionalized with gadolinium, to act as positive contrast agents (T1) for MRI. In order to explore the effect of protecting and coating the SPIONs a green chemistry procedure using extracts of Danube Delta leaves Nymphaea alba were applied in a separated sample fraction, adapting the procedure described by A. N. Dizaji *et al.* [4]. These leaves are considered a promising biocompatible, stabilizing and reducing agent. All the nanomaterials will be characterized in terms of size, stability, and morphology by a combination of techniques such as UV-vis, DLS, PXRD, FTIR, SEM, TEM, and Zeta Potential. The amounts of Fe and Au will be quantified by ICP-OES. Simultaneously, they are submitted to a detailed and systematic study by Mössbauer spectroscopy and magnetization studies, to compare both approximation methods in terms of their magnetic performance. Cytotoxic assays were also done to check their biological availability. Studies of hyperthermia and radiosensitization will be performed in view of their potential therapeutic properties.

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Lignin extracted from Acacia wood for the development of a sustainable hair conditioner

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Lignin is a natural aromatic polymer with good potential to be a renewable and sustainable feedstock. Its attractive properties justify the increased attention in the last years, being widely used for biomaterials development with broad industrial applications, such as an additive for cosmetic formulations [1]. Its complex molecular structure makes its extraction from the natural source quite challenging. For this, deep eutectic solvents (DES) have shown promising results and a ternary DES composed of choline chloride (ChCl) and two acid hydrogen-bond donors proved to be highly efficient and selective solvents for lignin extraction from biomass [2]. Our group tested different molar ratios of a new ternary DES (lactic acid: citric acid: choline chloride) and screened these mixtures for Acacia wood fractionation to inferabout their suitability for an efficient and selective extraction of lignin. A new solvent with “green” features was developed and the conditions for enhanced extraction were also identified. After the extraction process, the DES could be recovered and reused without compromising its efficiency (Figure 1), which provided a highly appealing process for lignin extraction from biomass.
Once having isolated this high pure lignin, cationic derivatives are being synthesized to be used as conditioners for hair care formulations. Hence, this work attains to develop new eco sustainable and biodegradable conditioners for hair care products using a natural and abundant source, like wood from Acacia, an invasive plant in Portugal, which will allow to overcome the negative impact synthetic conditioners have on aquatic organisms and the environment.

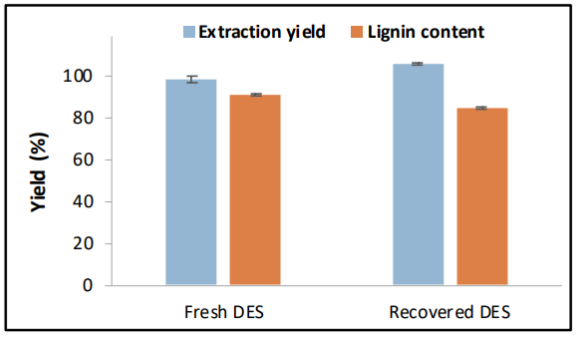

Figure 1. Extraction yield and purity of lignin using freshly synthesized and reused DES.

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GABA-modified squaraine dyes: the power of introducing amino acid units into potential PDT photosensitizers

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Squaraine dyes are a family of polymethine cyanines recognized for their intense absorption in the visible, namely in the red region, good chemical stability, and ability to produce reactive oxygen species in a biological environment after the incidence of an adequate light source, and as such, as potential photosensitizers (PSs) for photodynamic therapy (PDT) [1].

One of the primary deficiencies inherent in the PSs as a large is their limited solubility in water, explained by being generally composed of apolar-nature molecular structures [2]. Examples of methods reported to prepare more biocompatible potential drugs are the structural conjugation with polymers and molecules of a polar nature or by encapsulation in liposomes or polymeric particles [3,4].

This communication presents the synthesis of two squaraine dyes derived from 2,3,3-trimethylindolenine and 1,2,2-trimethyl-1*H*-benzo[*e*]indole with *N*-ethyl chains functionalized in the central ring with gamma-aminobutyric acid (GABA). The introduction of this biomolecule is mainly related to the polarity of this molecule, allowing it to modulate the typical lipophilicity of this core of dyes. PSs' water solubility values (logS) were predicted using *in silico* tools. Photostability was evaluated by performing absorbance measurements over light exposure, and singlet oxygen generation was assessed through 1,3-diphenylisobenzofuran assay. Regarding their biological interest, half-maximal inhibitory concentration values were determined for both compounds against three cell lines, two tumor cell lines (Caco-2 and PC-3) and one non-tumor cell line (NHDF), under irradiated and non-irradiated conditions. Light treatment was conducted using self-made light-emitting diode systems centered at specific and suitable emission wavelengths. For the most promising PS candidate, complementary experiments were carried out to understand better its action mechanism: colocalization studies, and evaluation of its genotoxicity, and subG1 population quantification and cell cycle arrest.

Squaraines' GABA-functionalization proved relevant in modulating their lipophilicity, formulating more biocompatible dyes. Biologically, the dyes exhibited remarkable activity, particularly for the prostate cancer PC-3 cell line, reaffirming the importance of studying the photodynamic activity of this class of dyes in the potential fight against oncological diseases.

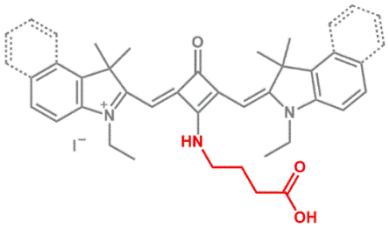

Figure 1. GABA-modified squaraine dyes involved in this communication.

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Computational design of new halogenated isoniazid derivatives - continuing the fight against tuberculosis

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Tuberculosis (TB) is the infectious disease with the highest number of fatalities in the world, after COVID, and its treatment, in most cases, is still based on isoniazid (INH), INH is still being used as a template for developing new compounds to fight TB. From a combination of experimental and computational studies, three series of INH derivatives were developed and tested [1,2]. However, the most promising compound series, the alkyl hydrazide series (INH-aC_n), which presented excellent *in silico* properties, seemed to be too unstable in the aqueous medium, leading to inferior antitubercular activities. [2]. In this work, we explored the role of halogenating the aliphatic derivatization to slightly deactivate the C–N bond and provide the well-needed stability to this compound series. For that purpose, we systematically added halogen in different positions of the lipophilic tail of INH-aC_4 and estimated the IN* formation reactivity of the final derivatives using Quantum Mechanics calculations [2]. The fluorine and chlorine derivatives showed promising reactivities, unlike the bromine derivatives which seemed to still be quite unstable. The second part of this work consisted of studying the most promising chlorine derivative interacting with the membrane via Molecular Dynamics simulations (Figure 1) [2]. We analyzed the membrane insertion, deformation, and total area variation of both the chlorinated and its non-chlorinated analog and showed that the halogenation of the alkyl tail does not perturb significantly the membrane interaction. This work allowed us to show that the design of halogenated INH derivatives may be key in the fight against tuberculosis.

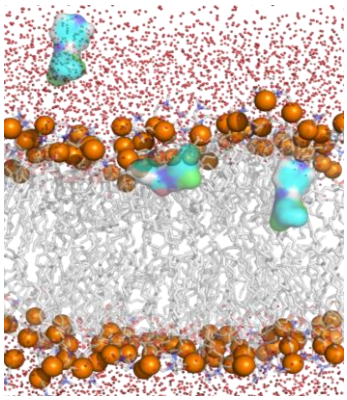

Figure 1. Illustration of membrane insertion of the INH derivative with a 4-carbon lipophilic tail and 2 chlorine atoms in the first carbon.

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Design and Synthesis of Siderophore-Antifungal Conjugates

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The problem of antifungal resistance to human fungal pathogens represents an increasing challenge in modern medicine. There is an imperative need to expand the antifungal armamentarium by researching alternative approaches to traditional therapy [1,2]. Recently, siderophores have opened new horizons to overcome this severe situation. This alternative approach has attracted great interest in medicinal chemistry and antimicrobial drug discovery but has yet to be applied to fungal infections [3]. The potential conjugation of siderophores with antifungals allows them to act as Trojan Horses by hijacking the microorganisms' highly developed iron transport systems to carry the antifungal agent into the cell and to deliver an effective and enduring response to multidrug-resistant pathogens [4].

Thus, the present work involves the design, synthesis, and lead generation of new siderophores antifungal conjugates for treating antifungal infections. First, a library of siderophores mimetics was achieved from 2,3-bis(benzyloxy)benzoic acid, a molecule frequently found in naturally occurring siderophores, and commercially available primary amines as building blocks. The newly synthetic siderophore units or known natural siderophores were conjugated with commercial and new/optimised in-house antifungal drugs through different coupling strategies. As a linkage between siderophore and antifungal, cleavable self-immolating linkers were constructed. The structure elucidation of all the synthesized compounds was established by nuclear magnetic resonance (NMR) spectroscopy.

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Mitochondria-targeted anti-oxidant AntiOxCIN$_4$ improved liver steatosis and cardiac metabolic alterations in Western diet-fed mice

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Being overweight or obese are among the main risk factors for developing non-alcoholic fatty liver disease (NAFLD), which is considered to be the most prevalent chronic liver disease worldwide. The prevalence of NAFLD has been increasing rapidly and coexists with metabolic disorders such as type 2 diabetes, hypertension, and cardiovascular disease. While the process leading to fat accumulation is well-documented, the pathophysiology of NAFLD/NASH is multifactorial, and the mechanisms underlying the progression to advanced forms remains unclear. The multifactorial pathogenesis of NAFLD/NASH poses a challenge to disease therapy. Although substantial efforts from scientific community and the large number of pre-clinical and clinical trials, there is still any FDA- or EMA-approved drug to specifically treat NAFLD/NASH. We previously developed a mitochondria-targeted anti-oxidant (AntiOxCIN$_4$) with remarkable anti-oxidant properties by conjugating caffeic acid anti-oxidant moiety with an alkyl linker and a triphenylphosphonium cation (TPP+).

In the present work, we hypothesized that AntiOxCIN$_4$ (2.5 mg/day/animal) may prevent hepatic and cardiac non-alcoholic fatty liver (NAFL) phenotype development in a C57BL/6J mice fed with 30% high-fat, 30% high-sucrose diet for 16 weeks. AntiOxCIN4 decreased body (by 43%), liver weight (by 39%), and plasma hepatocyte damage markers in WD-fed mice. Hepatic-related parameters associated with a reduction of fat liver accumulation (by 600%) and the remodeling of fatty acyl chain composition compared with the WD-fed group were improved, probably due to a AntiOxCIN$_4$-induced stimulation of hepatic fatty acid oxidation. AntiOxCIN4 also induced a hepatic metabolism remodeling by upregulating mitochondrial OXPHOS, anti-oxidant defense system and phospholipid membrane composition, which is mediated by the PGC-1α-SIRT3 axis. Histological analysis of cardiac tissue showed that either WD-fed and AntiOxCIN$_4$ supplemented mice did not present alterations in structural or inflammatory biomarkers. On the other hand, proteomic analysis showed that WD diet decreased the expression of several proteins associated with oxidative phosphorylation and estrogen receptor signaling, while increased protein expression associated with mitochondrial dysfunction, glycolysis and gluconeogenesis. Interestingly, AntiOxCIN$_4$ prevented the WD-induced alterations proteins associated with estrogen receptor signaling and mitochondrial dysfunction.

The amelioration of whole-body parameters in WD-fed mice and especially, a healthier phenotype of hepatocytes together with enhancement of cardiac mitochondrial function, supports the use of AntiOxCIN$_4$ as a potential candidate for the prevention/treatment of NAFLD-associated hepatic and cardiovascular complications.

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Mushrooms4Life: Decoding the Molecular Basis of an Anti-Cancer Small RNA Extracted from Edible Mushrooms

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Edible mushrooms have been used in human diets for thousands of years. They are considered functional foods and are an excellent source of nutraceuticals that play important roles in promoting human health and preventing diseases, such as cancer. However, our knowledge of the biocompounds responsible for these effects is still limited. Recently, ethanol-insoluble and water-soluble small RNA (sRNA) fraction purified from co-extracted polysaccharides by anion-exchange chromatography from some edible mushrooms species as *Cantharellus cibarius* (CCI) and *Boletus edulis* (BED). These sRNAs emerged as potent biomolecules, exhibiting the ability to induce apoptosis and inhibit cell proliferation in cancer cells without adverse effects on normal cells [1, 2]. As a result, there is a great potential for mushroom sRNAs to be used for cancer prevention and biotherapy.

To unravel the molecular nature of this sRNA, an extensive study was conducted, employing the previously reported extraction method [1, 2] for isolating this fraction from the previously characterized species as well as *Agaricus bisporus* Portobello (ABI). Additionally, microRNA extraction was performed from powdered mushrooms and High Molecular Weight Material (HMWM). The content of these fractions was analyzed for understand its composition and were tested in cancer and normal cells.

Preliminary data indicates that the presence of this sRNA fraction varies across mushroom species, as we were not able to detect the same fraction by anion-exchange chromatography in ABI. Notably, CCI exhibited the most promising results, demonstrating superior purity and a higher concentration of the sRNA fraction. Cell viability tests conducted on cancer and normal cells further underscored the immense potential of the sRNA isolated from CCI and BED, with significant anti-cancer effects observed at low concentrations without inducing cytotoxicity in normal cells. Furthermore, the cellular testing of miRNAs isolated from these species yielded no discernible effects on either cell line, emphasizing the likelihood of molecular enrichment within a specific ribonucleic acid sequence specifically targeting human cancer cells. This hypothesis gains support from the RNA-FISH analysis using a specific probe isolated from CCI3, which revealed hybridization signals in cancer cells treated with CCI and BED.

This work represents an essential preliminary step towards comprehending the molecular nature of this intriguing sRNA and its function within cancer cells. By delineating the molecular and functional profile of this sRNA, we can harness its promising anti-cancer properties and pave the way for future research and potential application of these sRNA biomolecules in cancer biotherapy.

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The role of tertiary amines in chromeno[3,4-*b*]xanthones for Alzheimer's disease

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Alzheimer's disease (AD), which is the most common form of neurodegenerative dementia, leads to memory loss as well as other cognitive functions impairments [1]. Currently, there are three drugs approved for AD treatment, and more recently two new drugs were approved by the fast-track approval of Food and Drug Administration (FDA), however, these compounds are only capable of alleviating certain symptoms and quality of life [2,3]. Therefore, the development of disease-modifying drugs to slow the progression of AD has become a global priority [3]. The fact that important targets in several cell signalling pathways can interact with one another to build a disease network is a distinctive and intricate aspect of AD [4]. As a result, single-target drugs, like the ones mentioned above, have a poor therapeutic effect [4]. Due to the complex pathogenesis of AD and the limitations of single-target drugs, in the last few years, multi-target strategies are emerging as a potential treatment for AD [4,5]. This is because one multi-target drug may have an impact on several targets related to AD, which may have a synergistic effect on the disease network and improve memory and cognition [6]. Herein, we describe the synthesis, NMR structural characterization, and biological evaluation of the aminated chromeno[3,4-*b*]xanthones towards the selected targets, cholinesterases (AChE and BChE) and β-amyloid (Aβ) aggregation (Figure 1).

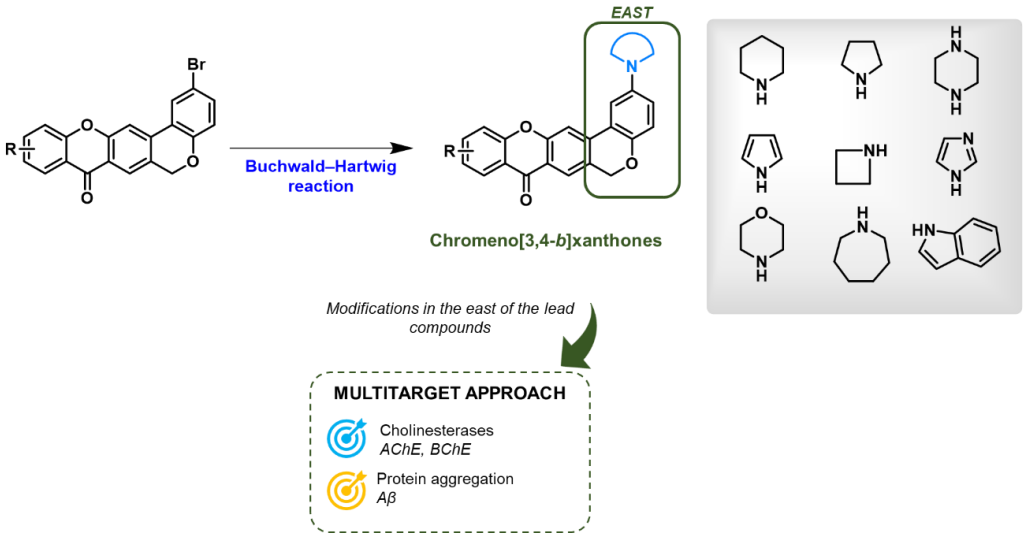

Figure 1. Aminated multifunctional chromeno[3,4-*b*]xanthones on the way to the multitarget compounds for AD.

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Hepatoprotective effect of Côa Valley (Portugal) plants' extracts in a Non-Alcoholic Fatty Liver Disease (NAFLD) cell model

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The Côa Valley region in the northeast of Portugal is home to over 500 plant species, most of them with potential medicinal properties. Among them, *Equisetum ramosissimum* Desf. has been used in folk medicine to treat hemorrhages, urethritis, hepatitis, and rheumatic diseases. On the other hand, *Rumex induratus* Boiss. & Reut. is an Iberian endemic herbaceous plant, typically consumed in this northern region, in salads and with mashed potatoes, plus being traditionally used to reduce inflammation and constipation [1]. Besides these plants, the genus *Geranium* spp. L. that comprises over 400 species of annual, biennial, and perennial plants, is represented in Côa Valley, by the species *Geranium purpureum* Vill. and *Geranium lucidum* L. being both reported as antipyretic agents, for pain relief, to stop hemorrhages, and to treat gastric and inflammatory diseases [2]. Given the immense potential of the medicinal plants that inhabit the Côa Valley area, this natural heritage demands to be preserved and valorized. Therefore, in this work four medicinal plants from this Portuguese northeast region were tested for their hepatoprotective effect on Non-Alcoholic Fatty Liver Disease (NAFLD).
Decoction and hydroalcoholic (EtOH 80%) extracts of *E. ramosissimum*, *G. lucidum*, *G. purpureum*, and *R. induratus*, were chemically characterized for their phenolic composition by HPLC-DAD/ESI-MSn and their cytotoxicity assessed using the Alamar blue® and sulforhodamine B® assays. To determine the hepatoprotective potential, HepG2 cells were previously incubated with the plant extracts and then subjected to palmitic acid (PA). The lipid accumulation was then determined through the Nile Red® assay.
E. ramosissimum and *R. induratus* extracts presented *O*- and *C*- glycosylated flavonoids, respectively, as the major phenolic compounds, while *G. lucidum* and G. *purpureum* presented ellagitannin derivatives (geraniin). The plant extracts studied, caused minimal cell metabolic and cell mass decrease at 25 and 50 µg/mL concentrations, indicating their safety. At 100 ug/mL, the cell metabolic activity was moderately affected in all plant extracts, apart from both *E. ramosissimum* extracts. Following PA incubation, the cell viability was not altered. Regarding the PA-induced lipotoxicity, pre-incubation with the decoction extract of *E. ramosissimum* significantly decreased neutral lipid accumulation. These findings suggest that the decoction extract of *E. ramosissimum* can significantly decrease the lipid accumulation on an *in vitro* cell model of NAFLD.

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Conjugating efflux pump inhibitors with siderophores: a novel approach to fight antibacterial resistance

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The emergence of microorganisms that are resistant to traditional antibiotics has risen to critical levels and is now considered a global health threat [1]. Therefore, the search for alternative approaches to the development of novel antibacterial agents has become a priority [2]. Promising strategies include the conjugation of antibiotics with moieties that will potentiate the antibacterial activity, resulting in target-directed new molecules [2]. In particular, the conjugation of siderophores with antibacterial agents has shown encouraging results, with a new drug recently approved [3,4]. The overexpression of efflux pumps constitutes one of the most common resistance mechanisms in bacteria [5]. For this reason, in this work, we aimed to synthesize conjugated compounds of siderophores/siderophore mimetics and bacterial efflux pump inhibitors (EPIs) to obtain novel molecules that might have potential in the fight against antibacterial resistance. We also aim to evaluate their antibacterial activity, potential synergism with other antibiotics and their capacity to inhibit bacterial efflux pumps.
Herein, several siderophore-EPIs conjugates were synthesized through the coupling of siderophore mimetics by a linker portion to promising EPIs. Structural elucidation of the compounds was obtained by nuclear magnetic resonance techniques (NMR). The assessment of the antibacterial activity of the synthesized compounds was performed *in vitro* against a panel of reference and clinically relevant bacterial strains, and the results of those assays will also be discussed.
Future work includes expanding the library of siderophore-EPI conjugates and a complete evaluation of the bioactive potential of the compounds.

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Synthesis of fluorinated carbohydrate derivatives with antibacterial potential against Gram-negative bacteria in combination with adjuvant agents

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The rapid emergence of difficult-to-treat infections caused by multidrug-resistant Gram-negative (MDR) pathogens poses a global threat to human health. Therefore, the discovery and development of new antimicrobial therapies in response to this growing concern has become a topic of utter importance [1]. In light of the biocompatibility of carbohydrates and their demonstrated value as antibiotic building blocks [2], this research work aims at optimizing the structure of alkyl deoxyglycosides — a class of innovative sugar-based bactericides able to disrupt phosphatidylethanolamine-enriched bacterial membranes [3]. Leads **1** and **2** (**Figure 1**) have additionally shown strong antibacterial activity against top-priority carbapenem-resistant Gram-negative clinical isolates when combined with subtherapeutic concentrations of the adjuvant agents colistin and polymyxin B [4]. As part of our structural optimization plan for these compounds towards improved antibiotic activity and reduced toxicity, the introduction of fluorine atoms in positions 4 and 6 of the carbohydrate scaffold (**Figure 1**) was thoroughly investigated. Importantly, owing to the highly electron-withdrawing properties of the fluorine atom, the selective bioisosteric replacement of hydrogen with fluorine atoms is expected to render new molecules with (i) enhanced membrane permeation; (ii) fluorine-promoted changes in dipolar interactions with potential for improved binding affinity to the biological target; and (iii) improved metabolic stability, among other advantages [5]. In this communication, we will disclose our latest results on regioselective DAST-promoted sugar deoxyfluorination, as well as optimized O- and C-glycosylation reactions towards the synthesis of new lead analogues for future biological evaluation in combination with colistin and polymyxin B against carbapenem-resistant Gram-negative bacteria.

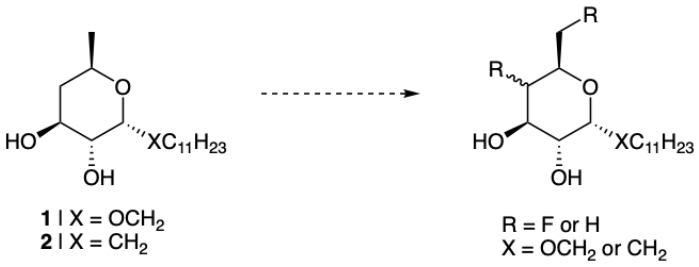

Figure 1. Lead analogues and target molecules.

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Edible coatings on grape-vine by-products infusions

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The wine industry is responsible for many environmental problems because of the large amounts of residues. However, several studies have shown that these wine industry residues, such as grapes, skins, seeds, and leaves are a rich source of nutraceutical compounds and present a great potential for developing new food products. Effectively, they represent a complex matrix of bio-compounds, such as phenolic compounds, flavonoids, procyanidins, anthocyanins, tannins, catechin, quercetin, kaempferol, transresveratrol, and nutrients such as vitamin C. Current studies have been stating that foods produced with wine and vine by-products or their extracts have antioxidant, anti-inflammatory, cardioprotective and anti-aging anti-cancer activities, etc. which are beneficial to human health. The increasing preference of consumers for natural food additives encourages the use of wine products as an alternative source of natural antioxidants in the food industry. However, due to processing (drying, mincing), some of the vine by-products are perishable and may present a short shelf-life. The protection of the developed products can be achieved through the utilization of edible films and coatings. For this purpose, this study aims to elucidate the different types of edible coatings that can be used in the preparation of grape by-products for foods and drinks, namely grape vine infusions made with dried minced grapes and dried minced grape pomaces. Besides the usually used coating material such as chitosan, Arabic gum, gelatin, and alginate, other compounds will also be used, namely soy lecithin, maltodextrin, inulin, and starch as well as their combination to create an edible film with high quality.

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Study of the sensorial and chemical properties of cookies supplemented with almond skin

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Almonds are one of the most produced nuts worldwide, and, during its production, several by-products are generated, which remain underutilized. One of those by-products is the almond skin, for which numerous studies have shown that they have nutritional and medicinal characteristics [1-2].
This work aimed to study samples of cookies supplemented with different amounts (0, 1, 2 5 e 10%) of almond skins. The cookies were evaluated for their total polyphenol content, antioxidant capacity, total flavonoids, *ortho*-diphenols, soluble sugars, starch, texture, and color. They were also sensorially evaluated, using a panel of tasters specialized in this type of evaluation and a QDA sensory test (Quantitative Descriptive Analysis test).
The results showed that the cookies with the highest levels of phenolic compounds, as well as the highest antioxidant activity (by the ABTS, DPPH, and FRAP methods), were the cookies supplemented with 10% almond skin. The total phenolic content obtained for the prepared extracts varied between 0.127 mg GAE/g and 0.415 mg GAE/g, the flavonoid content ranged from 0.067 mg CAE/g to 0.339 mg CAE/g and the *ortho*-diphenol contents varied between 0.163 mg ACE /g and 0.303 mg ACE/g, for the control cookie and 10%, respectively. Regarding the quantification of soluble sugars, the values were presented in percentage of fresh weight, and ranged from 30.148 to 38.054%, regarding the quantification of starch, the percentages varied from 14.488 to 21.982%.
Sensorially, and after applying a QDA test with a well-studied lexicon [3], we verified that the samples were statistically different in terms of the descriptors “Color”, “Roasted aroma” and “Dissolubility”, with a higher score in these descriptors for the cookies with 10% of almond skin.
This process of obtaining cookies, which can be industrialized, is interesting both from a nutritional point of view and for the possibility of creating new, differentiated, and innovative products.

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The potential synergistic effects of melatonin on polyphenolic profile and antioxidant activity of red wines

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Red wines deserve being paid special attention because contain a lot of bioactive compounds with antioxidant properties. Melatonin has a powerful antioxidant activity [1] and its presence in grapes and wines has opened an interesting perspective associated to the potential health benefits related to the moderate consumption of red wine. It is well known that phenolic compounds from wines exhibit positive influence on health status, acting as exogenous natural antioxidants. The health benefits attributed to a food/beverage do not depend on a single compound present in it, but combination of phytochemicals has been shown to enhance their bioactivities, by additive and synergistic effects. Melatonin can have a synergistic effect with other antioxidants, such as resveratrol [2]. These compounds naturally present in wine could act synergically to ensure a higher cytoprotective effect against oxidative stress, thus further supporting the hypothesis that health benefits of Mediterranean diet are partly due to wine [3].
The aim of this work is to study the potential synergistic effects, due to the application of melatonin in pre-stage of winemaking process for quality improvements of red wines with potential health benefits, on polyphenolic profile and antioxidant activity evolution. Therefore, Feteasca Neagra (FN) and Cabernet Sauvignon (CS) red wines were used and monitored after 3 and 6 months, respectively, from melatonin application in pre-stage of winemaking process. The red wines were obtained by two different winemaking processes, using punch, the process of breaking the cap and immersing in the must, 2 times a day for 7 days, as well as pumping over, the process of getting the juice from the soil of the fermentation tank and pump it over the cap, once a day for 7 days and treated with 3 different levels of melatonin (15, 30, 190 ng/mL). The electrochemical approach was paired with classical proper tools, spectrophotometer (ORAC and the TEAC assay) and chromatographic methods (HPLC with appropriate detection: MS and FL) to estimate the bioactivities of phytochemicals associated with additive and synergistic effects. All analysis were performed in key stages during the aging process of wines, both for the melatonin-enhanced red wines and for the control wines.
Following the application of melatonin, after 3 months, an approximately 20% increase in the concentration of anthocyanins (peonidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside) is observed compared to the concentrations found in the control wines, an increase observed in both FN and CS and which is maintained even after 6 months. Only in the case of malvidin-3-glucoside, the main anthocyanin in wines, small variations in concentrations of ±5% are observed. Melatonin induces increases in the content of polyphenols directly proportional to the applied concentration, especially in quercetin compounds (quercetin, quercetin 3-β-D-glucoside, quercitrin and rutin), (30% in FN and 10% in CS) and promotes catechin biosynthesis ((+)-catechin, (-)-catechin and (-)-epicatechin). Noteworthy are the values obtained for the concentration of resveratrol after 3 months, noting its biosynthesis which leads to an increase in the concentration of resveratrol by 50% in FN and 30% in the case of CS. The values obtained for ORAC and TEAC assay for antioxidant efficiency and total phenolics content (an 5% increase in FN and 10% in CS) are in agreement with those obtained for the electrochemical analysis of the resveratrol content, but also with those obtained for the quantitative HPLC-MS analyses of certain polyphenolic compounds, respectively anthocyanins.
Through HPLC-FL monitoring of the melatonin content at 3 and 6 months, it was observed the melatonin biosynthesis confirming that the alcoholic fermentation was not completed until 3 months.
After comparing the results obtained for the 12 red wines both at 3 months and at 6 months, it was decided to continue the study with FN obtained using punch and treated with 190 ng/mL of melatonin and with CS obtained using pumping over and improved with 15 ng/mL of melatonin.

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Characterization of Iberian Wine Vinegar: From flavour to health promoting compounds

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Wine vinegar is produced mainly in the Iberian Peninsula and has been widely used as a flavoring agent, a preservative and, in some countries, a healthy drink [1]. Wine vinegar is a rich matrix with manifoldness of nutrients and bioactive compounds that enhance flavour and has an essential role in preventing and treating human diseases through their antibacterial and anti-inflammatory properties [2] (Xia et al., 2020). This study aims explicitly to profile four categories of wine vinegar (white wine vinegar, red wine vinegar, balsamic vinegar, and Port wine vinegar) from the Douro and Rioja regions through sensory and chemical assessment techniques.

A descriptive technique, such as Quantitative Descriptive Analysis (QDA), was performed for a sensory profile. A trained panel of 15 panelists tasted twenty-two samples, fifteen from the Douro and seven from the Rioja. In the first stage, the panel was invited to do specific training on vinegar. Acetic acid solutions were prepared in aqueous and hydroalcoholic bases in different concentrations. The panel was asked to rate the samples considering an intensity scale (from less to most acid). Afterward, the panel assessed three standard samples, white wine vinegar (sample 1), red wine vinegar (sample 2), and Port wine vinegar (sample 3). The assessors freely generated sensory descriptive terms from these samples according to visual parameters such as color and clarity, olfactive parameters like smell and aroma, and taste parameters. A QDA test sheet with a 5-point scale (1-non detectable attribute to 5-clearly detected attributes, with intensity superior to the reference) was created based on a previous screening by reference frequency.

An extensive analysis was made for chemical characterization, considering several parameters, namely pH, color parameters, acetic acid, and ethanol concentrations, that were analyzed through an enzymatic kit. In addition, phenols content and antioxidant capacity were conducted through spectrophotometry. Moreover, the phenolic composition and amino acid content of vinegar samples were assessed by HPLC, and GC-MS determined the volatile compounds.

These techniques in symbiosis have been revealed to be a relevant, robust methodology to characterize wine vinegar, as it encompasses a dynamic and holistic perspective regarding complex matrices.

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Health-related compounds in Portuguese almonds

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Almonds are a rich source of nutrients, including proteins, fiber, healthy fats, vitamins, and minerals. Additionally, they contain a variety of bioactive compounds, including phytosterols and phenolic compounds, which have shown beneficial properties. Studies suggest that regular consumption of almonds may be associated with a range of health benefits, such as reducing the risk of cardiovascular diseases, reduction of oxidative stress and inflammation, and protection of cells from damage caused by free radicals. Almond is one of the most important nut worldwide, and in Portugal, latest data show that almond was the major nut produced, surpassing chestnut, mainly due to the new orchards being installed in the Alentejo region. These orchards are based in foreign cultivars, even though Portugal possesses an extensive list of traditional cultivars. However, these cultivars are poorly characterized, either considering the agronomical traits of trees, but also chemical composition of fruits. Hence, this work intended to characterize the chemical composition of Portuguese almonds (cvs. Casanova, Molar, Pegarinhos, and Refêgo), and comparing it to well-known foreign cultivars (French cultivar Ferragnès and Spanish cultivar Glorieta), focusing on compounds that are related to health benefits. For this, sucrose and α-tocopherol contents, as well as the phenolic and fatty acid profiles were studied. For all parameters, significant differences between cultivars were recorded. Sucrose content was, in average, lower in Portuguese cultivars, while, in contrast, α-tocopherol content was higher in those samples. Nineteen different phenolic compounds were found, with eight of them present in all samples. The sum of all individual phenolics was found to be higher in the foreign Ferragnès (3833 µg/100 g) and in the Portuguese Molar (3817 µg/100 g) than in the other cultivars, and the lowest content was recorded for Rêfego (1436 µg/100 g), being catechin the most abundant compound in all cultivars. Twenty-one fatty acids were identified in the almond cultivars studied; with oleic, linoleic, and palmitic acids being the major fatty acids. Portuguese cultivars presented improved health lipid indices, namely reduced atherogenicity index (lower in cv. Pegarinhos) and thrombogenicity index (lower in cv. Molar).
Portuguese almonds present interesting content in health-related compounds, comparable to foreign ones, and their characteristics should be exploited, either in raw form, but also in processed products, considering their specific features.

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Health-promoting potential of pear pomace

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The pear juice pressing process produces a significant amount of pomace of good nutritional quality, which is a by-product that raises problems of waste management along with economic and environmental issues [1]. Therefore, researchers have been focusing on finding valuable bioactive compounds from these wastes in which a great number of phytochemicals still exist, minimizing the waste burden and providing new sources of bioactive compounds.
Pomace powder after two-step extraction is characterized by higher content of phenolic compounds, including flavonoids, higher antioxidant, antidiabetic, antihypertensive and antibacterial activity. Moreover, the use of higher granulation pear pomace powder provided detection of TPC and TFC at a higher concentration, which directly resulted in high antioxidant activity (FRAP). The lower granulation pear pomace powder was more effective in anti-diabetic (as α-amylase and β-glucosidase inhibition), anti-hypertensive (as ACE inhibition) and antimicrobial (toward *E. coli* and *S. aureus*) activities. These results provide the first database in the literature on the potential use of pear pomace in accordance with the concept of sustainable development, representing a potential for future *in vivo* research.

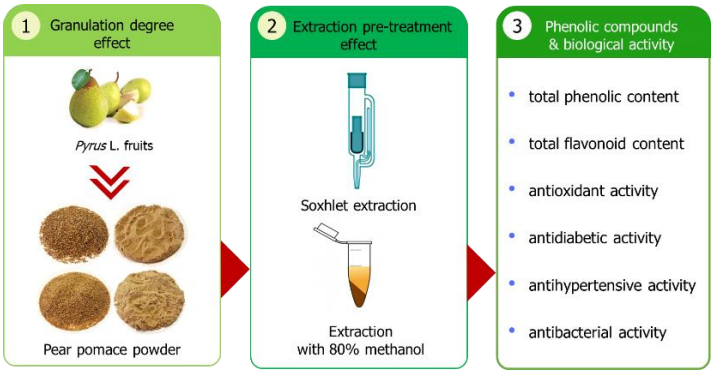

Figure 1

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Impact of different zones and seasons on theaflavins contents and biological properties of Azorean *Camellia sinensis* samples

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Tea from *Camellia sinensis* (L.) is being used from centuries as a health drink, and is an important source of bioactive compounds, the majority of which are polyphenols that are known to have antiviral properties [1].The molecular constituents of *C. sinensis*, in particular epigallocatechin-3-gallate (EGCG) and, more remarkably, the galloylated theaflavins, mainly theaflavin-3,3'-di-*O*-gallate (TF-3,3'-DG), have been reported to inhibit SARS-CoV-2 3CL-protease, an enzyme required for the cleavage of its polyproteins to produce vital individual functional proteins used for viral cell replication [2]. The aim of the present study was to investigate the phenolic (TPC), flavonoids (TFC), TF-3,3'-DG, and total theaflavins contents of *C. sinensis* black tea in different seasons and in different zones of Gorreana Tea Plantation, for further optimization of the horticultural and processing conditions to maximize its biological activity. The determination of theaflavins was achieved by HPLC following the Matsubara and Rodriguez-Amaya [3] methodology. The TPC and TFC contents were determined by colorimetric methods, with some modifications [4]. The results are presented in Table 1 and revealed higher values for all samples in summer season, particularly for TPC that presented a significant difference between spring and summer seasons. Zone C presented the highest values of TPC, TF-3,3'-DG and total theaflavins, however, Zone A revealed higher values of TFC in summer season related to Zone B and C. This study also showed that altitude of plantation zones influences the levels of tea components, particularly the levels of polyphenol oxidase (PPO), and consequently the formation of theaflavins (e.g. higher in Zone C). This can be explained by the fact that pH of the soil decreased with higher altitude influencing the PPO levels. This study revealed the possibility to create a novel Azorean antiviral tea, investigating the best tea plantation zones that shows higher biological properties.

Table 1. Total phenolic and flavonoid contents, theaflavin-3,3'-di-*O*-gallate and total theaflavins content of *C. sinensis* black tea samples in different seasons and collecting zones of Gorreana Tea Plantation[a].

	Zone A		Zone B		Zone C	
	Spring	Summer	Spring	Summer	Spring	Summer
TPC (mg GAE/g DE)	154.87 ± 3.88	244.65 ± 1.21	172.04 ± 2.49	259.37 ± 1.46	180.76 ± 2.31	272.59 ± 0.25
TFC (mg RE/g DE)	96.03 ± 0.24	136.31 ± 4.45	99.22 ± 0.87	108.25 ± 1.50	101.31 ± 0.86	112.98 ± 1.73
TF-3,3'-DG (mg/g DW)	2.19 ± 0.02	2.51 ± 0.02	2.84 ± 0.03	3.78 ± 0.04	2.71 ± 0.02	4.43 ± 0.06
Total theaflavins (mg/g DW)	11.41 ± 0.06	12.58 ± 0.05	14.88 ± 0.18	15.31 ± 0.16	12.42 ± 0.09	16.27 ± 0.13

[a]Values are mean ± SD (*n* = *3*). GAE, Gallic acid equivalents. RE, Rutin equivalents. DE, dry extract. DW, dry weight. TPC, total phenolic content. TFC, total flavonoid content. TF-3,3'-DG, theaflavin-3,3'-di-*O*-gallate. TF+TF-3-G+TF-3'-G+TF-3,3'-DG, total theaflavins. The tea samples were collected in the following altitudes of tea plantation (Zone A: 205 m, Zone B: 228 m, and Zone C: 341 m, above the sea level).

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Anti-biofilm and Anti-Adhesive effect of polycationic polyurea dendrimers (PURE) against *Listeria monocytogenes*

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Bacterial biofilms, as a natural strategy of microbial defense, are a major public health problem which still lacks an effective solution. Biofilms are extremely resistant to the action of antimicrobials, making urgent the development of alternative agents. *Listeria monocytogenes*, which is the main cause of listeriosis, is one of the most concerning infections related to the formation of bacterial biofilms. Since it can be found in raw foods and refrigerated products, consumed without prior heating, the presence of this bacteria presents a higher risk for the consumer. Here we explore for the first time the effectiveness of polycationic polyurea dendrimers (PURE-OEI) against *Listeria monocytogenes* biofilms. In this sense, we decided to analyze the potential of a polycationic dendrimer (PURE-OEI), design by us, against *Listeria monocytogenes* bacteria. This bacterium is known to respond poorly to conventional therapies as we evaluated here. Also, to unravel the dendrimer mechanism of action and its potential against *Listeria monocytogenes* we performed multiple assays including determination of MIC, live-dead assay using confocal microscopy and electron microscopy (SEM) to evaluate the morphological changes of biofilms. Bacterial cell adhesion assays at a high initial density 1x10^8 CFU/mL here also explored. The results obtained point towards a high efficiency of PURE-OEI in the treatment of *L. monocytogenes* infections. We also found that polycationic core-shell dendrimers have an impact on cell density and biofilm adhesion, which could lead to a potential application against the formation of *L. monocytogenes* biofilms. Finally, using SEM it was possible to observe that PURE-OEI display a disruptive action at the bacterial membrane level. As we previously demonstrated [1], polycationic dendrimers presents a possible solution to fight antimicrobial resistance. Based on our results, we suggest that combined traditional antimicrobial therapies with polycationic dendrimers can be a potential efficient treatment. Also, our data can open the way to the creation of strategies for preventing *L. monocytogenes* primary infection (e.g. development of smart packaging by incorporation of PURE-OEI).

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Biological properties and L-theanine, a stress reliever amino acid, from Azorean *Camellia sinensis* on different plantation zones

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One of the reasons that *Camellia sinensis* green tea has been worldwide used for centuries is its relaxation benefits and human health properties due to the presence of polyphenols, and particularly L-theanine, an amino acid found primarily in green tea that produces calming effects in the brain, improve memory function, and has influential impact on diseases, such as: diabetes, hypertension, cardiovascular disorder, tumor suppression and also stimulates the generation of alpha-waves that cause a relaxation sense [1,2]. Determination of L-theanine (L-THEA) was achieved by HPLC following the Baptista *et al*. [1] methodology, and epicatechins derivatives (ECD´s) followed by Baptista *et al*. [3] methodology. The total phenolic (TPC), flavonoid (TFC) contents and free radical scavenging activity (FRSA) were determined by colorimetric methods, with some modifications [4]. The aim of this study was to show the variation of L-THEA and polyphenols in Azorean *C. sinensis* green tea from different collecting zones of Gorreana Tea Plantation. The results are presented in Table 1 and revealed that the highest values of L-THEA were observed in Zone 3, followed by Zones 1, and 4 and the lowest value in Zone 2. However, Zone 2 presented the highest levels of TPC and TFC, and Zones 3 and 4 presented the lowest level of TPC and TFC, respectively. For FRSA the results were very similar between zones with slightly better results in Zone 4. For ECD´s the results were also very similar between zones, presenting higher values in Zone 1 and 2. In conclusion, Zone 2 has higher levels of TPC, TFC and ECD´s, however, shows lower levels of L-THEA. This can be explained by the fact that theanine is produced in tea plant root, travel through the plant and under the effect of sunlight is used as a skeleton to the catechin formation. According to previous studies of the authors [4], the tea chemical composition may reflect the influence of relevant factors (e.g. genetic strain, geographic origin, climate conditions, and volcanic soil) and consequently, different tea quality may be produced from different tea plantation zones.

Table 1: The L-theanine, total phenolics, flavonoids contents, epicatechin derivatives contents and free radical scavenging activity of Azorean *Camellia sinensis* green tea samples in different collecting zones of Gorreana Tea Plantation[a].

	Zone 1	Zone 2	Zone 3	Zone 4
L-THEA (mg/g DW)	14.31 ± 0.14	7.16 ± 0.58	16.36 ± 0.24	12.26 ± 0.42
TPC (mg/g GAE)	291.43 ± 4.38	294.99 ± 3.23	275.70 ± 4.01	283.76 ± 3.62
TFC (mg/g RE)	42.53 ± 0.64	53.07 ± 1.14	42.93 ± 0.76	38.80 ± 0.72
ECD´s (mg/g DE)	217.39 ± 0.72	217.41 ± 3.05	212.78 ± 2.21	208.35 ± 0.38
FRSA (EC50 - µg/mL)	5.38 ± 0.09	5.89 ± 0.28	6.24 ± 0.02	5.07 ± 0.11

[a]Values are mean ± SD (n = 3). GAE, Gallic acid equivalents. RE, Rutin equivalents. DE, dry extract. DW, dry weight. EC50, Half-maximal effective concentration. L-THEA, L-theanine. TPC, total phenolic content. TFC, total flavonoid content. Epicatechin derivatives (ECD´s), EGC+EC+EGCG+ECG. FRSA, free radical scavenging activity. The tea samples were collected in the following altitudes of tea plantation (Zone 1 - 205 m; Zone 2 – 212 m; Zone 3 - 235 m, and Zone 4 - 341 m, above the sea level).

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Composition, nutritive value, and bioactivity of five edible algae sold in the Portuguese market

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Macroalgae have been introduced in human and animal food due to their nutrients and relevant properties such as antioxidant, antitumor, antiviral, and antimicrobial activity. Thus, their incorporation in the formulations of various products, such as functional foods, is a result of their potential benefits on the human health, promoting economy through blue biotechnology. However, they also have bioremediation capability being able to retain in their cells toxic compounds present in the culture medium, so when transformed into food these compounds will be present in the final product. Several studies point out the presence of contaminants in algae, which may invalidate the health benefits and the preventive effects on humans [1,2].

The present study aimed to evaluate the elemental composition by Energy Dispersive X-ray Fluorescence (EDXRF) of the following edible algae species, with different origins, sold in the Portuguese market: two green algae *Undaria pinnatifida* (Wakame from China and Japan) and two brown algae *Laminaria longissimi* (Kombu from South Korea and Japan), and a red alga *Porphyra yezoensis* (Nori from China). The bioactivity of the compounds presents in seaweed samples, were also evaluated, by determining the contents of polysaccharides (neutral, sulfated, *β*-1,3-D-glucans), phenolic compounds and proteins, as well as their antioxidant potential [2,3].

The analysis of potassium, chlorine, sulfur, and calcium by EDXRF revealed that the highest values were observed for *Laminaria longissimi*. However, for all the studied samples, the presence of total arsenic was observed, ranging between 26 and108 ppm.

Aqueous extracts from the seaweed samples were obtained by using autoclave (water; 121 ºC; 30 min) and multistep extraction (with cold and boiling water, acidic and alkaline conditions) methods. Alkaline fractions generally showed the highest content of the evaluated biomolecules and antioxidant activity. Since the biological activities of polysaccharides are correlated with their structure, FTIR spectroscopy was used carry out structural analysis of these compounds in the aqueous extracts.

Antibacterial activity of the obtained extracts was assessed against *Escherichia coli* and *Staphylococcus aureus* as well as their *in vitro* cytotoxicity towards mammalian cell lines.

There is no doubt that these functional foods are good sources of nutrients, however the lack of strict control measures in several countries should alert consumers to the indiscriminate use of these food products.

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The therapeutic effect of Korean Red Ginseng extract and Epimedium Koreanum Nakai on ulcerative colitis

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Ulcerative colitis (UC) is a generally experienced digestive organ sickness in the contemporary world that ends into colorectal disease; consequently, the convenient therapy of UC is of central issue. Panax ginseng Meyer is a widely consumed natural product in South East Asian nations, particularly Korea. It shows many naturally useful characteristics for nearly head-to-toe afflictions in the body. Epimedium koreanum Nakai (EKN) is likewise a broadly utilized customary Korean natural medication utilized for treating barrenness, stiffness, and cardiovascular diseases. Separately the calming exercises of both red ginseng extracts (RGEs) and EKNs had been shown in the past in different fiery models; be that as it may, we tried to disentangle the mitigating effects of these two concentrates in dextran sulfate sodium (DSS)- induced ulcerative colitis in mice model on the grounds that the allopathic solutions for UC include more secondary effects than benefits. Our outcomes have shown that the blend of RGE + EKN synergistically lightened the naturally visible sores in DSS-actuated colitic mice, for example, colon shortening, hematochezia, and weight reduction. In addition, it reestablished the histopathological sores in mice and diminished the degrees of favorable to fiery arbiters and cytokines through the restraint of NF-κB and nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP-3) articulation. *In vitro*, this mix additionally diminished the size of nitric corrosive (NO), supportive of provocative arbiters and cytokine through NF-κB and mitogen-enacted protein kinase (MAPK) pathways in RAW 264.7 mouse macrophage cells.

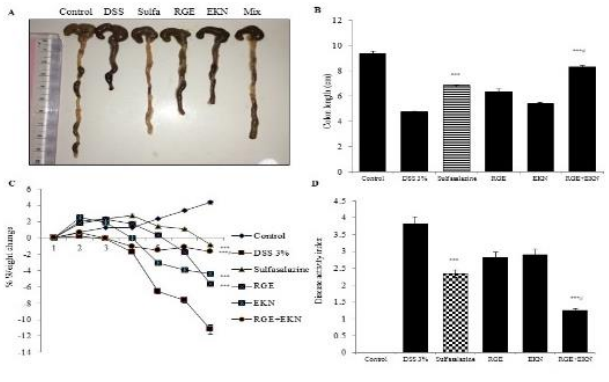

Figure 1. Revival of colon length and DAI by Rg3-RGE+EKN treatment. C57BL/6 mice were given 3% DSS in drinking water for 7 days along with *p.o* treatment of the extracts. After 7 days, mice were euthanized and the colon tissue extracted for macroscopic and microscopic lesion assessment. (a) Gross examination of colon length; (b) Quantification of colon length; (c) % Change in the body weight of mice; (d) Disease activity index (DAI).

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Unpacking the impact of high-fat diet on liver antioxidant response and mitochondrial bioenergetics to microplastic-induced toxicity

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Microplastics are identified as one of the top emerging global environmental problems [1]. The European Chemicals Agency classifies microplastics as 'extremely persistence' in the environment, which can lead to non-reversible pollution, presenting a potential risk to the environment and/or human health [2]. Despite the common perception of microplastics as solely an environmental issue, recent findings suggest a complex web of pathways contributing to food, water, and air contamination. As a result, human exposure to microplastics has become increasingly prevalent, challenging the traditional view of this problem [3]. Nowadays, it is recognized that microplastics may present a hazard to human health. Nonetheless, there is no scientific evidence regards the adverse effects that microplastics may present once in the human body and if lifestyle factors can influence the toxicological outcomes. Animal models present the best approach to investigate the possible mechanisms underneath the possible toxicity that this contaminant may present to human health. Using an animal model, this work aimed to understand the impact of different dietary regimens in microplastic toxicity on liver antioxidant response and mitochondrial bioenergetics. Male ICR mice (5-week-old) were divided into two main groups and fed either a standard diet or a high-fat diet (standard diet with 20 % (v/w) of sugar cane molasses and 10 % (v/w) of olive oil). Later on, each group was subdivided into two. One was fed the correspondent dietary regimen with microplastics resulting from mechanical degradation of single-use plastics items incorporated (0.01 % (w/w), 90 – 190 nm). Thus, four experimental groups were evaluated: STD (animals fed with a standard diet without microplastics); STD+MPs (animals fed with a standard diet without microplastics); HFD (animals fed with a high-fat diet without microplastics); HFD+MPs (animals fed with a high-fat diet with microplastics).

Animals fed with a standard diet containing microplastics present oxidative damage, as seen by the increased malondialdehyde levels. Enzymatic antioxidant defenses were differently affected by the presence of microplastics, with glutathione reductase presenting an increased activity ($p=0.0354$), whereas catalase and glutathione-S-transferase activity were inhibited ($p<0.0001$ and $p=0.475$, respectively). These results show that microplastics lead to hepatic oxidative stress, even though the cellular redox status, evaluated through the glutathione content reduced and oxidized, was maintained, probably due to the increased glutathione reductase activity. Oxidative stress was also present in animals fed a high-fat diet with microplastics, even though the lipid peroxidation levels remained constant, probably due to the increase observed in catalase activity ($p<0.0001$).

Additionally, serum biochemical analysis revealed increased alanine transaminase levels, showing liver damage. The mitochondrial respiratory function remains unchanged in both dietary regimens with or without microplastics, once when hepatic mitochondria were energized with succinate (enzymatic substrate of complex II), the oxygen flux of the respiratory state 3 and 4 remained similar. These results point out that high-fat diets may exacerbate the toxicological effects of microplastics, corroborating the necessity to address the impacts of lifestyle factors in risk analysis on microplastics' toxic outcomes.

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Plant bioactive compounds analysis for coagulation/filtration enhancement

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The wine industry is attained as one of the most important socio-economic activities in the world. In order to keep the quality of the wine, it is necessary to proceed to sanitation processes during all stages of production, leading to the consumption of large volumes of water, which is been considering nowadays essential, considering the drying weather that has been verified in several countries like Portugal. In addition, the wastewater generated in sanitation process has a high organic content, most of which is very recalcitrant and can't be degraded by biologic processes.
Thus, in this work, it was analyzed the bioactive compounds of a group of plants (pollen from *Acacia dealbata Link* (A.d.), seeds from *Chelidonium majus* L. (C.m.), *Daucus carota* L. (D.c.) and *Tanacetum vulgare* L. (T.v.) and rachis from *Vitis vinifera* L.), with the aim to enhance the treatment of a real winery wastewater (WW) by coagulation/filtration.
The Fourier-transform infrared spectroscopy (FTIR) analysis showed bands at 3352.21 cm^{-1} attributed to stretching vibrations of OH groups from water, alcohols, phenols, carbohydrates, peroxides, fatty acids, and lignin. Thus, the next step involved the fatty acids analysis by Gas Chromatography with Flame Ionization Detection (GC-FID). Results showed high amounts of fatty acids in the composition of D. c. seeds (17136 µg g^{-1}) and low concentration in rachis (2806 µg g^{-1}). Among the fatty acids in D. c. seeds, it was shown major percentages of saturated fatty acids capric and undecanoic, and unsaturated fatty acids myristoleic and palmitoleic. A phenolic differentiation was performed by high-performance liquid chromatography with photodiode-array detection (HPLC-DAD), showing high content of phenolic acids in A. d. pollen ((3038442 µg g^{-1}) and low concentration in rachis (144796 µg g^{-1}). Among the phenolic compounds, HPLC-DAD allowed differentiation of 4-hydroxybenzoic acid, epicatechin, ellagic acid and oleuropein. With the extraction of the compounds from the plant parts using NaCl solution (1 M), it was applied as a coagulant to a real WW with a DOC = 400 mg C L^{-1}, turbidity = 298 NTU and total suspended solids (TSS) = 750 mg L^{-1}. The coagulation-flocculation-decantation (CFD) was optimized, by (1) variation of pH and coagulant dosage, (2) variation of mixing speed, (3) variation of flocculant type and (4) variation of flocculant concentration. As flocculants, it was used enological-based such as bentonite, activated charcoal, potassium caseinate and polyvinylpyrrolidone. Scanning electron microscopy (SEM) analysis revealed high porosity between the particles, thus more organic carbon could be adsorbed by these agents. Afterwards, a filtration (F) was applied to the wastewater and sludge volume index (SVI) and sludge volume reduction (SVR) indicated the sludge was of good quality (SVI < 100 mL/g). In addition, a fouling reduction analysis showed that using plant extracts (PEs), reduced up to >99% the fouling of membranes, which meant a cost reduction for wastewater treatment plants (WWTP). A phenolic extraction and analysis by HPLC-DAD showed a significant reduction of the phenolic content of the real WW, with exception for wastewater treated with A. d. pollen and T. v. seeds, which showed significant increase in phenolic concentration, a fact related with the high phenolic content present in the plant parts.
Thus, based in these results, it is concluded that PEs + enological-based flocculants are a sustainable and efficient source to reduce the organic carbon, turbidity and TSS from real WW.

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Use of natural anaesthetics for the management of fish stress with a view to sustainable production and welfare improvement

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In intensive aquaculture, practical procedures are frequent, which can induce stress and influence their welfare. To minimize these negative effects, which can have significant economic impacts, the use of anaesthetics is increasingly recommended. Tricaine methanesulfonate (MS-222) is a synthetic anaesthetic often used in aquaculture and research, however some studies support this compound to induce further changes in fish welfare. In this sense, it is necessary to find alternative anaesthetic compounds that are harmless to the fish, the environment, and the consumer with the purpose of enable safe and sustainable fish production. The zebrafish is a teleost fish which has been widely used as a research model being a representative of commonly produced species in aquaculture. The larval stages of zebrafish (from 72 hpf - hours post-fertilization - onwards) are highly suitable for attending welfare mechanisms as, at these stages, the brain structure is well developed and showing stressful stimulus–response properties. Therefore, the objective of this study was to evaluate different anaesthetic compounds to minimize the stress induced by a stressful stimulus.

To this end, 96 hpf zebrafish larvae were relocated to 50 mL centrifuge tubes with a final volume of 20 mL of E3 medium containing different anaesthetic compounds (MS-222 (150 mg L^{-1}), eugenol (80 mg L^{-1}), thymol (15 mg L^{-1}) and menthol (50 mg L^{-1})) and E3 as the control group. Tubes were shaken for 1 min at 250 rpm. Samples were collected at different times (10 min, 1 h and 4 h) after the stimulus. Control samples without the stress stimulus were euthanized with an overdose of MS-222. Samples were then assessed for changes in cortisol, lactate and glucose levels, energy consumption, ATPase activity and DNA damage by spectrophotometric methods.

Regarding the results, cortisol, lactate, and glucose levels were not different within treatments after 10 minutes or 1 h. However, after 4 h of the stimulus, cortisol and glucose were lower in the eugenol and menthol groups compared to the control group. These results show that the compounds have the capacity to minimize the stress markers in the animal after a period of 4h. At 10min and 1h probably the compounds had not yet been totally absorbed by the animal.

There were no differences between treatments in the energy consumption from resazurin oxidation, DNA damage, or ATPase levels.

Considering the results, these natural anaesthetics compounds seem to be secure an minimize the effects caused by a stressful event in fish. Moreover, although further molecular studies are needed, no physiological changes were observed thereby supporting their use for sustainable production and fish welfare improvement.

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Elderberry (*Sambucus nigra*): A Potential Antigenotoxic Food for Promoting Health

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A healthy diet can play a vital role in maintaining good health. Elderberry, a nutritious food with potential genoprotective effects, has been linked to improved skin health due to its antioxidant and anti-inflammatory properties. In this study, we investigated the antigenotoxicological effects of elderberry using the comet assay in *Drosophila melanogaster* neuroblasts and human lymphocytes. Four concentrations of elderberry (1%, 5%, 10%, and 15% (w/v)) were tested in both assays, using hydrogen peroxide and streptonigrin as DNA-damaging agents. Our findings indicated that elderberry has potential as an antigenotoxic agent and may help protect skin cells from environmental damage caused by UV radiation and pollution [1]. In recent years, the concept of "nutraceuticals" has gained popularity as an approach to promote health and prevent disease by using food-based products with medicinal properties. Elderberry is one such food that has been shown to have potential as a nutraceutical due to its high content of bioactive compounds like anthocyanins, flavonoids, and phenolic acids [2]. These compounds have been linked to a range of health benefits, including antioxidant, anti-inflammatory, and immune-boosting effects [3]. In addition to its potential as an antigenotoxic agent, elderberry has been studied for its potential in treating various conditions, such as respiratory infections, cardiovascular disease, and even cancer [4-6]. Therefore, consuming a healthy, balanced diet can promote overall health and maintain good skin health. The integration of nutraceuticals, such as elderberry, and cosmetics represents an exciting advancement in the field of skin health and beauty. By combining the power of internal nutrition with external cosmetic interventions, this approach offers a comprehensive strategy for enhancing the complexion and promoting overall skin well-being. These ingestible cosmetics can deliver a range of nutrients and antioxidants that support cellular health, combat oxidative stress, and reduce inflammation. Simultaneously, cosmetic products play a vital role in topical skincare, offering targeted solutions to address specific skin concerns and enhance appearance. By synergistically utilizing both nutraceuticals and cosmetics, individuals can optimize their skincare routine, benefiting from the combined effects of internal nourishment and external application, leading to healthier, more radiant skin. Overall, the findings of this study suggest that elderberry can potentially help protect skin cells, making it a promising natural alternative for genoprotection.

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Promoting bioactive composition and antioxidant properties of sweet cherries through biostimulant strategies

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Sweet cherries are increasingly chosen by consumers due to their exceptional organoleptic properties, high nutritional value, and well-established health-promoting attributes. The remarkable bioactive activities of this fruit, closely linked to its abundant content of phenolic compounds with antioxidant properties, have earned it widespread recognition. Several strategies are used to enhance cherry quality, with the latest efforts being placed on biostimulants. Biostimulants, as promising environmental-friendly products, have emerged with the goal of promoting the overall sustainability of production systems, while maximizing productivity and improving the nutritional value and quality of fruits, increasing their concentration of phenolic compounds and antioxidant activity.

Thus, this work intends to highlight the effect of the application of two concentrations of seaweed-based *Ecklonia maxima* (SW 0.30% and SW 0.15%) and glycine betaine (GB 0.40% and GB 0.25%) biostimulants and their combination (Mix- SW 0.15% + GB 0.25%), on the bioactive content (total phenolics and flavonoids) and antioxidant traits (DPPH, FRAP, and ABTS) of fruits from cultivars 'Early Bigi' and 'Lapins'. Sweet cherries were harvested from a commercial sweet cherry orchard, grafted on "Saint Lucia 64" rootstock, located in Resende, Northern Portugal. The treatments were foliar applied at three different phenological stages of fruit development (stages 77, 81, and 86 according to the BBCH scale). To achieve more accurate results, treatments were applied for three consecutive years (2019, 2020, and 2021). The results only refer to the last year of application of biostimulants. Overall, the total phenolic and flavonoid contents presented an increase, for both cultivars, with the application of the SW 0.15% and the MIX treatment. For cv. 'Lapins', the same pattern was observed for antioxidant activity by DPPH and FRAP methods, with the application of SW 0.15% and Mix treatment, although for ABTS method no significant differences were observed when applying biostimulants. Regarding cv. 'Early Bigi', best antioxidant results were recorded when Mix treatment was applied to the fruits.

Although cherries already exhibit a substantial content of bioactive compounds, the exogenous application of biostimulants enhances its concentration, thereby adding further value to this fruit.

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Functional Properties of Bioactive Compounds from *Porphyridium cruentum* and their Application in Food Industry

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The significant development of microalgae biotechnology has made these microorganisms powerful "cell factories" for functional food production, leading to a rapid growth of the algal bioeconomy in the food and feed industries. Microalgae biomass is considered one of the most highly sustainable sources, presenting advantages over terrestrial biomass such as higher growth rate, low water consumption, carbon neutral emissions, and production of several bioactive compounds, such as proteins, pigments, polysaccharides, lipids and phenolic compounds [1].

This communication aims to present the results concerning the production of intra and extracellular bioactive compounds by *Porphyridium cruentum* under autotrophic conditions and different nitrogen concentrations (6 mM and 18 mM NO_3^-) at laboratory scale and in flat panel photobioreactors (FP PBR) (Fig. 1). To isolate intracellular biocompounds (IBC) the harvested biomass at the beginning of the stationary phase was subjected to multistep extraction (MSE) (with cold and boiling water, acidic and alkaline conditions) and the fraction obtained with HCl that was identified as having high polysaccharide content was further fractionated by ion-exchange chromatography. Concerning the extracellular biocompounds (EBC) solubilized in the culture medium, these were recovered by ultrafiltration. The isolated IBC and EBC were quantified by colorimetric methods and their antioxidant activities were also investigated. Structural analysis of the obtained polysaccharides was achieved by FTIR spectroscopy, and photophysical properties of phycobiliproteins were attained by UV-Vis and fluorescence spectroscopy [2,3].

Overall, the biomass grown at 6 mM NO_3^- demonstrated higher IBC contents and the produced IBC and EBC have demonstrated a huge potential to be used in challenging areas, such as food engineering, due to their biochemical properties.

Figure 1. Growth of *P. cruentum* cultures in (**A**) lab scale and (**B**) flat panel photobioreactors.

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Influence of Process and Chemical Composition of Chestnut on Sensory Profile and their Effect on Consumer Acceptability and Health

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The chestnut has become an important food ingredient in the area of nutrition and gastronomy [1]. Its chemical and nutritional properties allow it to change the food quality and to sensorially enrich several traditional dishes, including desserts. Studies indicate that chestnuts contain small amounts of crude fat, but contain monounsaturated fatty acids (MUFAs) which are known for their anti-cancer and cardiovascular effects [2]. In addition, they also point out that it is low in carbohydrates, but the amount needed for long-term and short-term energy [3]. This study focused on the evaluation of the crude fat content, soluble sugar, and starch content of processed chestnuts - thawed, roasted, and boiled - of the Judia and Longal varieties. Besides, a CATA (check-all-that-apply) test was performed on consumers to understand the influence of processing on the sensory quality of the chestnuts. The results effectively highlight the low crude fat content, and, regarding carbohydrates, starch showed a higher concentration than soluble sugars. The sensory test revealed that roasted chestnuts are strongly characterized by their aromatic attributes, while thawed and boiled chestnuts are described by their texture attributes. This suggests that thawed chestnuts may be characterized by their crunchiness, while the attribute that stands out in the boiled ones is their dissolubility. Therefore, incorporating chestnuts or other nuts into our diets can provide a healthier option for consumers who want to improve their overall health and well-being. However, it's always important to keep in mind that no single food can provide all the nutrients your body needs, and a varied and balanced diet is the key to good health.

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Enzymatic reduction of sugar content in sucrose-rich fruit products

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Sugar consumption is a major concern in modern society, especially in developed countries. Sugar is essential to our organism, although when intake is excessive, it can lead to the development of diseases such as dental caries, obesity, Type-2 Diabetes Mellitus, cardiovascular diseases and metabolic dysregulation [1]. A common source of sugar in our diets is fruit. While consumption of whole-fruits has a more complex metabolization in the body, sugars from fruit-derived products, as fruit juices and concentrates, are easily absorbed. These products are consumed directly or added to other foods and present higher concentrations of sugars. Here we aim to find a solution to this problem, with the Clean Label concept in mind. Clean Label foods, although not legally defined, are currently a rising trend among consumers, driven by health, sustainability, and environmental concerns. This concept aims the production of foods that are minimally processed, organic, “natural” and free from artificial ingredients, with the shortest possible list of ingredients [2]. Our objective was to reduce the content of sugar in sucrose-rich fruit concentrates recurring to the enzymes invertase and fructosyltransferase, capable of convert sucrose into fructooligosaccharides (FOS), which are low caloric, with sweet taste, contribute to satiety and body weight control, have low glycaemic index, are non-carcinogenic and are well-known prebiotics [3]. Enzymatic applications are mostly classified as processing aid, as they are not functional in the final products, and do not need to be labeled. We applied the enzymes at a concentration of 12 U/g of initial sucrose at endogenous pH of samples, varying conditions of temperature (10 and 35 ºC) and time (0,2,4,6,24 h). Results showed that both enzymes are capable of caloric reduction of at least 50%. This reduction results from conversion of sucrose into FOS. This conversion mainly occurred early in the reaction, slowing conversion rate over-time. Interestingly, the reaction can be carried out at low temperature and at the endogenous pH of sample without significantly changing it. These early results show promise as a Clean Label solution to reduce sugar content in sucrose-rich fruit products and are a promising starting point for future work.

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Effect of pre-harvest application of biostimulants on phenolic content and antioxidant activity in blueberry (*Vaccinium corymbosum* L.)

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Blueberry (*Vaccinium corymbosum* L.) is a fruit recognized for its attractive organoleptic and nutritional characteristics. This fruit is an excellent source of bioactive compounds and is known for having strong antioxidant activity, which leads to the prevention of various diseases [1, 2]. Due to these characteristics, blueberry production and commercialization have been increasing over time [3]. However, as the fruit industry faces global challenges, adopting pre-harvest strategies, such as the use of biostimulants marketed as eco-friendly alternatives, can be a sustainable way to improve blueberry production and quality. The objective of this research was to evaluate the effect of pre-harvest foliar application of glycine-betaine (GB) and *Ecklonia* maxima macroalgae (EM) biostimulants on the phenolic composition (total phenolics, ortho-diphenols, flavonoids, and total anthocyanins) and antioxidant capacity (by CUPRAC, DPPH, and FRAP methods) of 'Duke' and 'Draper' blueberry cultivars. The study was conducted in an orchard located in Vilarandelo, Portugal, where seven treatments were administered at three different stages (full bloom, early green fruit, and fruit colouring), including a control treatment (T0). Two different doses of a commercial EM-based product, at 4 L/ha (T1) and 2 L/ha (T2), a GB-based product, at doses of 4 kg/ha (T3) and 2 kg/ha (T4), and the combination of 4 L/ha EM + 4 kg/ha GB (T5) and 2 L/ha of EM + 2 kg/ha GB (T6) were applied. Foliar spraying with a high dose of EM (T1) and a low dose of GB (T4) increased the total phenolics content of cv. Duke significantly, when compared to T0. Furthermore, 'Draper' blueberries treated with a high dose of GB (T3) showed a higher content of total phenolic compounds, and exogenous T3 application increased total anthocyanins content in both cultivars. The foliar application of the high dose of EM (T1), a low dose of GB (T4), and both doses of the two biostimulants (T5 and T6) increased total anthocyanins content in 'Draper' fruits, and T5 and T6 improved antioxidant activity in cv. Duke using the CUPRAC and FRAP assays. The antioxidant activity of both cultivars increased following the use of GB (T3), as measured by the CUPRAC method. In addition, 'Draper' blueberries treated with EM (T1 and T2), GB (T4), and with the combination of EM + GB (T5), according to the CUPRAC assay, registered a significant increase in antioxidant activity. This study found that the use of glycine-betaine and algae-based biostimulants can be a sustainable strategy for improving the nutritional value of blueberries.

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Almond by-products as an alternative source of phenolic compounds and antioxidant activity

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In the production and industrial processing of almonds, large amounts of by-products are generated, which can represent more than 70% of the production volume. These by-products are mostly misused but can be exploited due to their recognized bioactivity [1-3]. In this study, the bioactive composition (total phenolics, flavonoids and ortho-diphenols) and antioxidant activity (by the ABTS, DPPH and FRAP methods) of four by-products (hull, shell, skin and blanching water) from the fruits of four almond tree cultivars (cvs. Ferragnès, Ferraduel, Marinada and Lauranne) were evaluated. The results point to a significant influence of the cultivar in all parameters under study. In the case of hulls and skins, all parameters showed higher values in cv. Ferraduel. For shells it was cv. Ferragnès that stood out, while, regarding blanching water, there were statistically significant differences in the content of total phenolics and flavonoids and in the antioxidant activity by the DPPH and FRAP methods. In this case, it was cv. Lauranne that presented higher values in all parameters evaluated, with the exception of antioxidant activity by the FRAP method where the highest values were recorded in cv. Ferragnès. In summary, this work showed that almond by-products are rich in bioactive compounds and that the cultivar has a decisive influence on their composition. Thus, in order to increase the value of these by-products, sustainable and eco-friendly alternatives that enhance their use must be developed.

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Green Synthesis of Luminescent Carbon Nanomaterials from *Porphyridium cruentum* Microalgae

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Carbon dots (CDs), an innovative type of carbon-based nanomaterial have fascinated the scientific community due to their good biocompatibility, excellent optical properties, cost-effective, environment-friendly synthesis methods, abundant functional groups, minor cytotoxicity and remarkable photostability, finding widespread application in fluorescent bioimaging and nanomedicine, chemo/biosensing, photocatalysis and optoelectronics devices [1-3].

The synthesis of CDs has been explored using a great diversity of green low-valued resources (*e.g.,* waste biomaterials, industrial waste, and agricultural biomass) and its use demonstrated [3-7].

Microalgae are a widely distributed, element-rich natural biomass exhibiting short growth cycles and high photosynthetic efficiency. And, as they can be obtained from natural water blooms or produced industrially, they are also a high-potential source for the synthesis of CDs [8,9].

Herein we report our most recent results concerning the μAlgae-carbon nanodots (μAlgae-CNDs) synthesis from industrially produced *Porphyridium cruentum*, by a one-step hydrothermal process, where the effect of temperature, additives, and residence time on the luminescence properties of μAlgae-CNDs were explored.

The structural characteristics of the as-synthesized μAlgae-CNDs were evaluated by FTIR and ^{1}H NMR spectroscopies and their photophysical properties investigated by UV-Vis and fluorescence spectroscopies. The μAlgae-CNDs exhibit blue-green fluorescence, significant quantum yield and a high photostability. To better understand μAlgae-CNDs potential clinical use, their in vitro cytotoxicity against four cell lines was evaluated, whose first outcomes are presented.

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Sensitive determination of phenolic compounds using a Pt-GO composite modified screen-printed electrode of simple preparation

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A significant number of bioactive molecules incorporate phenolic groups in their structure. These include natural antioxidants (*eg*. resveratrol, alfa-tocopherol, cathequine), medicines (*eg.* acetaminophen, propofol, dienestrol), hormones (*eg.* adrenaline, estradiol) and neuroransmitters (*eg.* dopamine, serotonine), among others. The electroactivity of these molecules is often related to the electro-oxidation of the phenolic moieties. Thus, the efficiency of this reaction determines the sensitivity and limits of detection of the electroanalytic quantification of these bioactive molecules [1]. The detection of these molecules is relevant in different contexts, including pharmaceutical formulations, biological fluids, foodstuff and environmental samples.

The use of screen-printed electrodes became highly attractive, due to the size and portability of the sensors (that include the working, reference and auxiliary electrodes) as well as the requirement of low volumes of sample. Nevertheless, the inks used for the electrodes printing incorporate non-conductive components (polymeric binders) that decrease their performance, with higher redox potentials and lower currents, leading to a decrease in sensitivity and increase of the detection limits [2].

A methodology for the modification of a screen-printed carbon electrodes to improve the sensitivity of the detection of phenolic compounds is presented. The modification process is simple, comprising the dropcasting of a suspension containing GO (graphene oxide) and H_2PtCl_4 at the electrode surface, followed by electrochemical reduction to form the nanocomposite Pt-ERGO (electrochemical reduced graphene). The extent of reduction and the Pt content was optimized for the sensor sensitivity towards hydroquinone (HQ), used as a phenolic model compound. The significance of this electrode modification is demonstrated by the improvement of the HQ detection sensitivity. The modified SPCE (ERGO-Pt@SPCE) was successfully used for the detection of HQ and BPA (bisphenol A) in mineral and tap water samples. The performance parameters of the detection of HQ and BPA are listed in Table 1.

Table 1. Performance parameters of the ERGO-Pt@SPCE for the detection of HQ and BPA.

		HQ	BPA
Sensitivity (µA/ µg mL^{-1})		15.3	1.93
Precision (%)*		4.6	7.6
Limit of detection (µg mL^{-1})		1.4	4.6
Recovery (%)#	*10 µM*	113	98
	20 µM	98	104

* Calculated as the method standard deviation (n=6)
# From spiked mineral water samples

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Aptamer-modified nanoparticles as biocatalyst

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Natural enzymes are extremely efficient at catalyzing a huge variety of reactions with high substrate specify, activities and yields under mild reaction conditions. As a result, there has been a significant interest in using them for diverse applications. However, problems as low operational stability, sensitivity to environmental conditions, difficulties in recovery and recycling limit their applications. To overcome these limitations great efforts have been made to explore efficient mimetic materials of these natural enzymes. Although nowadays it is well established that nanomaterials mimicking natural enzymes, nanozymes, posse several distinct advantages over natural enzymes as well as other reported artificial enzymes, they still face several limitations. Aptamers are sequence-specific nucleic acids exhibiting selective binding properties towards low molecular-weight substrates and macromolecules to which a catalytic unit can be tether. In this work the covalent linkage of aptamer binding sites to nanozymes, "aptananozymes", is introduced as a versatile method to improve the selectivity and catalytic activity of nanozymes by concentrating the reaction substrates at the catalytic nanozyme core, thereby emulating the binding and catalytic activesite of native enzymes. The concept was exemplified with the synthesis of Prussian blue (PB) nanozymes, functionalized with the *L*-hydroxy arginine binding aptamer for the H_2O_2-mediated oxidation of *N*-hydroxy-*L*-arginine to *L*-citrulline. The aptananozymes reveal enhanced catalytic activities as compared to the separated catalyst and respective aptamer constituents. This system provides nitric oxide (NO), which influences a number of critical physiological processes such as cardiovascular control and neuronal signalling. Due to these diverse biological roles, significant efforts have been devoted to exploring its potential as a therapeutic agent, where it has demonstrated antitumor efficacy and protection against cardiovascular injuries. The limitation it presents is that, as a gas, NO is unstable in vivo and is difficult to administer clinically in a controlled and continuous manner, which would be solved by generating NO in vivo as a byproduct with our system.

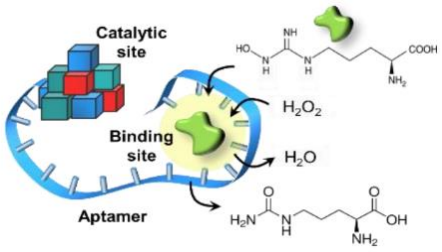

Figure 1. Schematic model of the catalytic "aptananozymes".

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Evaluation of New Indolenine-Based Squaraine Dyes as Potential Human Serum Albumin Fluorescent Probes

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The quantitative determination of proteins is an essential parameter in biochemistry, biotechnology and immunodiagnostics, and the reputation of serum albumin in clinical diagnosis should be highlighted, given that alterations in its concentration are generally associated with certain diseases. As possible probes for this purpose, squaraine dyes have been arousing the interest of many researchers due to their unique properties, such as strong absorption in the visible spectra, moderate relative fluorescence quantum yields and increased fluorescence intensity after non-covalent binding to specific ligands. In this work, four new squaraine dyes (Fig.1), were synthesized, characterized and evaluated *in vitro* concerning their potential application as fluorescent probes for human serum albumin (HAS) detection. The fluorescence assays performed with constant dye concentrations (2.0 μM) in buffer phosphate and increasing HSA concentrations (0.0 to 3.0 μM), revealed a good interaction between each dye and HSA, by the linearity observed between the concentration of HSA and the fluorescence intensity of the squaraine–protein solutions obtained from at the wavelength of maximum emission.
Finally, it was possible to conclude about the dye that provides the best response, and therefore, which is the one that has the best fluorescent probe attractiveness.

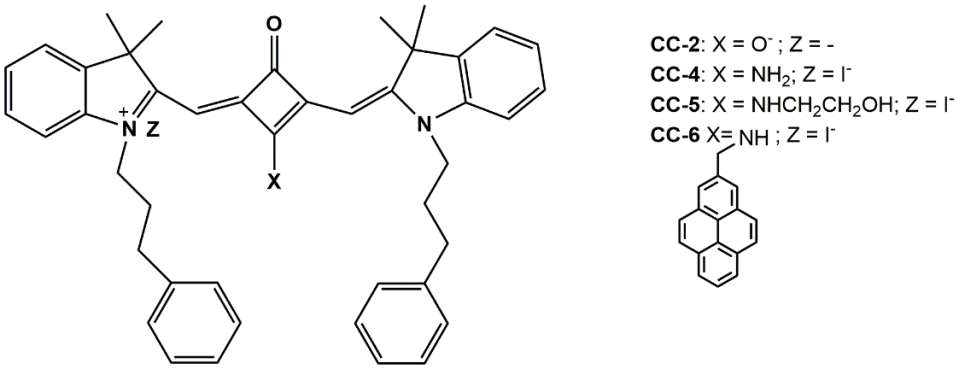

Figure 1. New squaraine dyes evaluated as potential probes for HSA detection.

Advanced fluorescence characterization of biomass-derived carbon nanodots

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Fluorescent carbon nanodots from biomass (B-CNDs) were synthesized using wet pomace (the semi-solid residue from the olive oil productive process) as the starting material [1], at 250 ºC for 72 h in a closed vessel (40 bar of pressure). The B-CNDs have bright blue fluorescence (QY = 0.20). The origin of such fluorescence is still a matter of research in our group. Steady-state and time-resolved fluorescence anisotropy was used to gain a deeper insight on the features of the emissive entities in our B-CNDs. Herein we report a study that puts together time-resolved fluorescence anisotropy, AFM and TEM of the B-CNDs to address the sizes and morphologies of the fluorophores. Time-resolved experiments were carried out in water-glycerol mixtures of different viscosities, starting from 0.97 cP (0 wt% glycerol) up to 5.70 cP (50 wt% glycerol), allowing one to estimate the effective radius of the emitters via the Stokes-Einstein equation. The results indicate a bi-exponential behavior for the anisotropy decays, with 123 and 1440 ps as correlation times in water, which translates into average particle diameters of 1 and 2 nm, which are in good agreement with the lateral size (1.6-4.6 nm; average 2.59 nm) obtained from TEM and height (0.5-4.6 nm; average 1.0 nm) from AFM measurements (see Figure 1). Results will be compared with those described in the literature [2].

(a) (b) (c)

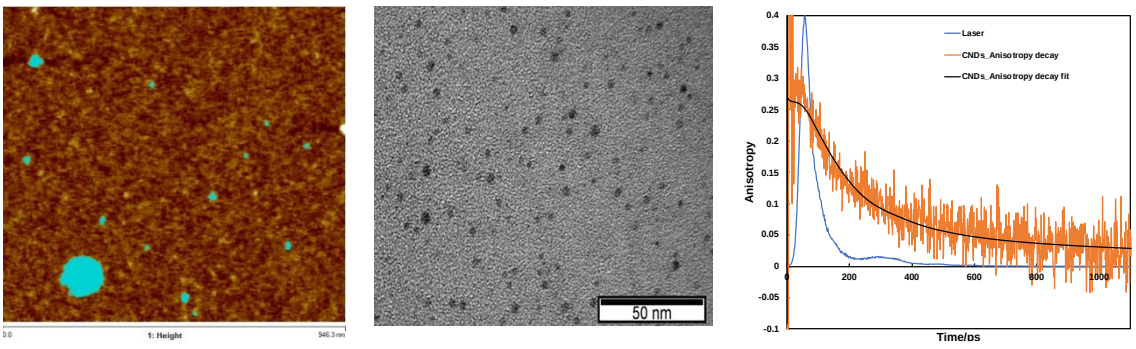

Figure 1. (a) - AFM image of the B-CNDs; (b) TEM image of the B-CNDs; and (c) - Bi-exponential anisotropy decay of and aqueous dispersion of B-CNDs in water at 0.001 mg/mL.

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Protein-based photonic hydrogel for sensing microRNAs

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Biopolymers are versatile materials with good biocompatibility, biodegradability, ease of manufacture and low production costs. Due to these properties, biopolymers are used in various bioengineering and biomedical fields, including biosensors [1]. Optical biosensors can be developed based on photonic crystal structures that reflect a certain color due to the periodicity of materials with different refractive indices in the wavelength range of visible light. Thus, the combination of stimuli-responsive natural biopolymer hydrogels with photonic structures can bring great advantages for real-time and label-free detection of analytes [2]. MicroRNAs are small non-coding RNA sequences involved in the post-transcriptional gene regulation, and they are important biomarkers for the early detection of various diseases, especially cancer [3]. Therefore, their quantification using biosensors can be a promising tool for non-invasive sensing. In this work, a novel composite hydrogel was developed by combining the proteins bovine serum albumin and NeutrAvidin (NAv). Chemical crosslinking through the formation of amide bonds together with heat-induced gelation resulted in hydrogels with good optical and mechanical properties. A three-dimensional photonic crystal made by self-assembly of colloidal poly(methyl methacrylate) particles was embedded in the protein hydrogel to create an optical transduction platform. In addition, incorporating the NAv protein (functional protein) into the hydrogel formulation allowed to easily modify it with biotinylated oligonucleotide probes to detect the target microRNAs. The detection and quantification of the microRNAs was based on measuring the changes in the reflectance spectra of the photonic hydrogels. The combination of biopolymers with structural colors benefits from the biocompatible and biodegradable properties of the natural biopolymers with the stimuli-responsive properties and label-free optical response of photonic hydrogels [4]. Hence, this protein-based photonic biosensor is a promising, simple, and cost-effective design for detection of miRNAs.

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The profiling of PAH biomarkers in wastewater from the point of view of the risk assessment of public health exposure to hazardous chemicals in urban areas

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There is a lack of knowledge and understanding of the local exposure to organic air pollutants, especially in highly urbanized regions of Eastern Europe. PAHs entering the body in a certain part are removed in unchanged form, but usually undergo several steps of biotransformation and detoxication process and are excreted as hydroxymetabolites through urine and feces. There is strong evidence of health risks associated with human exposure to organic air pollutants such as PAHs (polycyclic aromatic hydrocarbons) generated in combustion processes (solid fuels, coal, biomass) and communication. Most high-molecular PAHs are degraded only slowly and therefore represent a long-term potential health hazard to living beings. The aim of the study was the determination of the hydroxyl PAH metabolites profile in wastewater. The stability of three OH-PAHs in wastewater was confirmed in the first step of our studies and was presented in J. Durak *et al*. [1]. The 24 composite samples of wastewater influent were collected at the “Plaszow” wastewater treatment plant (WWTP) in Krakow, in winter and summer. This plant is the biggest one in the city and the third in the country, treating over a 70% of Krakow wastewater from over 480 thousand of inhabitants in the central part of the city, with an average capacity of 165.000 m3/day. Studies were carried out using SPE-GC-MS/MS method for the determination of selected OH-PAH (1- hydroxy and 2-hydroxynaphthalene (1-OH-NAP, 2-OH-NAP), 2-hydroxy and 9-hydroxyfluorene (2-OH-FLU, 9-OH-FLU), 9-hydroxyphenathrene (9-OH-PHEN), 1-hydroxypyrene (1-OH-PYR), 3- hydroxybenzo(a)pyrene (3-OH-BaP)) in samples collected in WWTP. Analysis was carried out by means of a Thermo Scientific GC TRACE 1300 (GC-(IT)MS/MS) and a TriPlus RSH Autosampler. The average concentrations of compounds ranged from 5 ng/L to more than 400 ng/L. The highest concentrations of OH-PAH were detected in the influents, for 2-OH-NAP and 9-OH-FLU in winter and summer. However, their concentrations were twice lower in summer. A similar trend was observed for other compounds. 1-OH-PYR was observed for influent and effluent samples only in winter at average concentrations of 8 ng/L and 5 ng/L, respectively.

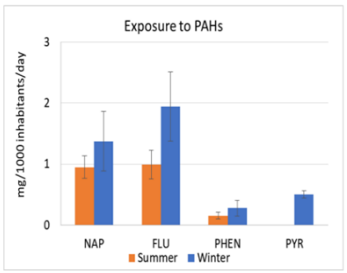

Figure 1. Average exposure to PAHs in winter and summer.

According to the research theses, information on the concentrations of polycyclic aromatic hydrocarbon metabolites obtained from wastewater may allow the assessment of exposure of the population to PAHs. In the summer period, an inhabitant of Krakow could absorb on average 2.1 µg of the tested PAHs per day, while in winter 4.1 µg. Differences between the summer and winter seasons may result from the variability of PAH concentrations in the air. These results can be considered as similar in terms of order of magnitude to the values presented in the work by Bojakowska *et al.* [2], where the total daily exposure to PAHs is estimated at 3 µg/day.

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Comparative Study of Tomato Waste Carbon Dots Bioactivity: Conventional Heating *vs.* Microwave Irradiation

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Tomato (*Solanum lycopersium*) is one of the most appreciated fruits due to its appetizing flavour and its various shapes and colours. With a production originating in South America, it stands out as a source of bioactive compounds and for its health-promoting properties. In 2021, around 4.8 million hectares of tomato were cultivated, representing a world production of approximately 182 million tonnes. In Portugal, one of the most important economic sectors is the tomato processing industry, reaching an annual production of *ca.* 1.4 million tons [1,2]. From this activity results the generation of high content organic tomato waste (TW), consisting mainly of seeds, vascular tissues, and peels. This waste, due to its reduced biological and oxidative stability, represents a problem with serious impacts on global warming and climate change, making its exploitation crucial [2,3].

The use of agricultural and industrial biomass as precursors for the synthesis of carbon nanodots (CNDs) has emerged as an alternative for the valorisation of abundant low-cost carbon resources, which associated with the easiness of its synthesis through simple and inexpensive methods, has attracted interest from the scientific community [4-7]. The optical properties, biocompatibility and low toxicity of these nanomaterials allow the diversification of their applications, in areas such as pharmacology, with particular interest in microbial control, imaging and photocatalysis [8].

Herein, we report the synthesis of CNDs from TWs following simple and sustainable one-pot conventional hydrothermal carbonization (HTC) and microwave-assisted irradiation (Mw-HTC), using ethylenediamine (ED) as nitrogen source. FTIR and ^{1}H NMR were used for structural characterization and optical properties were assessed by UV-Vis and fluorescence spectroscopies.

In order to evaluate the influence of the synthetic method on CDs bioactivity, several studies regarding antioxidant capacity and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* were performed. Preliminary results concerning CNDs cytotoxicity towards various cell lines are also presented.

Acknowledgments: We thank Sugal Portugal for tomato waste supply.

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An electrochemical sensor based on carbon xerogels for detection of dopamine

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Carbon xerogels (CXs) are porous materials that consist of a reticular structure formed by interconnected nanoparticles and are obtained through a sol-gel synthesis involving the polycondensation of hydroxybenzenes (e.g., phenol, resorcinol) and aldehydes (e.g., formaldehyde, furfural), where the obtained gel is dried under ambient temperature and pressure, followed by a carbonization process. CXs have attracted considerable attention due to their highly customizable textural properties that can be tailored for specific applications [1]. As a result, they are utilized in a wide range of applications, including energy storage and conversion [2], adsorption [3] and catalysis [4] making them a versatile option among the vast array of available carbon materials.
Dopamine (DA) is an important neurotransmitter in the human central nervous and hormone system and therefore participate in many physiological processes in the human body. However, imbalances in the levels of DA (too low or too high) might cause serious illnesses such as Parkinson's disease or schizophrenia [5]. Thus, a real-time and accurate detection of DA is desirable and vital for the diagnosis and treatment of neurological disorders.
In this communication, we demonstrate the effect of the presence of metals in a carbon xerogel matrix in enhancing the sensitivity of the detection of biomolecules containing phenolic groups. Hydroquinone (HQ) was used as a model polyphenol to characterize the electrochemical activity of CX and CoNir@CX with a sensitivity of 2.77 and 3.32 A M^{-1} cm^{-2}, respectively, estimated from cyclic voltammetry. For DA detection these materials exhibited a sensitivity of 2.09 and 1.55 A M^{-1} cm^{-2} estimated by differential pulse voltammetry for the CX and CoNir@CX, respectively.

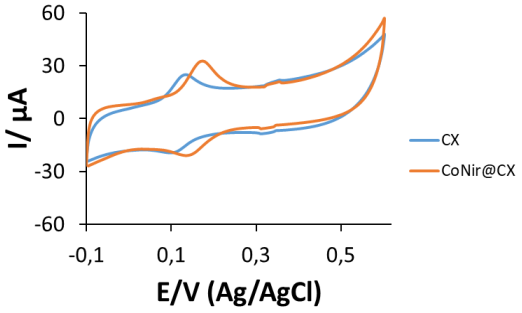

Figure 1. Cyclic voltammograms of CX/GCE and CoNir@CX/GCE recorded at 20 mV s^{-1} in 0.1M PBS, pH 7.0 containing 500 µM DA.

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The theoretical description for aesculetin and quercetin cathodic electrochemical determination in wines

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Douro wine and is one of the symbols of Portugal. Douro wine region was the first demarked in XVIII century. It has its own characteristic scent and flavor, due to the presence of some aromatic lactones. But the main alimentary value to the vine is given by its polyphenolic composition [1].
On the other hand, the chestnut C. Sativa is one of the most important product and ingredient for the cuisine of Trás-os-Montes. The districts of Vila Real and Bragança produce 25% of Portuguese chestnut. Its pulp and flowers also possess high concentrations of flavonoid and coumarinic polyphenols, mainly those with hydroquinonic moiteies, which, in quinonic forms, act as antioxidants and conservants, being thereby candidates for substitution of sulfite in wine preparation. Another positive feature of those compounds is their contribuition to polyphenolic wine profile, reason why the development of an efficient method for their determination is actual, and the electroanalytical method, being applicable to polyphenolic compounds, may be an interesting solution.
In this work, the possibility of cathodical electroanalytical determination of aesculetin and quercetin, the the most representative polyphenolic coumarin and flavonoid is theoretically described. The electroanalytical process is carried out on mildly acidic pH, correspondent to wine and may be exposed as on the Fig. 1.

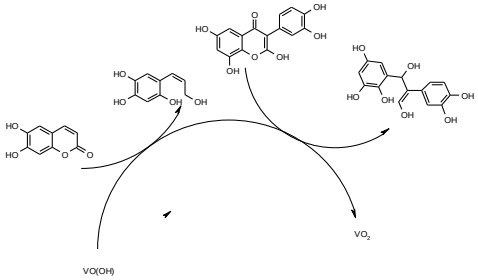

Figure 1. The schematic representation for electroanalytical process.

The system´s behavior will be described by a trivariant balance equation mathematical model (1):

$$\begin{cases} \frac{da}{dt} = \frac{2}{\delta}\left(\frac{\Delta}{\delta}(a_0 - a) - r_{11}\right) \\ \frac{dq}{dt} = \frac{2}{\delta}\left(\frac{D}{\delta}(q_0 - q) - r_{12}\right) \\ \frac{dv}{dt} = \frac{1}{V}(r_{11} + r_{12} - r_2) \end{cases} \quad (1)$$

And the analysis of the model by means of linear stability theory and bifurcation analysis confirms the efficiency of this method as itself and in comparison, with anodic electrooxidation of the same compounds.

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Development of polyester-based dendritic structures loaded with cisplatin for targeted treatment of osteosarcoma

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Osteosarcoma (OS) is a form of bone cancer that predominantly affects individuals under the age of 19. Despite advancements in treatment in recent decades, the survival rate for OS is still below 60%, and the rate decreases to 20-30% when metastasis or recurrence takes place. One of the primary drugs used for chemotherapy treatment of OS is cisplatin, nevertheless it comes with significant side effects, tumor recurrence, and frequently leads to permanent disability in patients [1,2]. Aiming at the achievement of a more efficient and less deleterious treatment of OS, we are developing bone-targeted and controlled drug delivery systems based on polyester dendritic structures with different functionalized peripheral groups and having the ability to be loaded with cisplatin. These systems (and their precursors) were synthesized and then systematically characterized through multiple techniques, including nuclear magnetic resonance (NMR), mass spectrometry (MALDI-TOF), scanning electron microscopy with Energy Dispersive X-Ray Analysis (SEM/EDX), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FT-IR), size exclusion chromatography (SEC), and dynamic light scattering (DLS). The in vitro biological activity of these systems is also being evaluated using relevant cell line models, namely their cytotoxic behavior before and after drug loading. In this regard, cell metabolic activity is being used as an indirect method of assessing cell viability, and information on cell morphology is being obtained by optical microscopy.

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Electrospinning and electrospray: potential alternatives towards improved photocatalytic systems

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Nowadays, electrospinning and electrospray are considered one of the versatile techniques for the manufacture of polymeric nanofibers and nanospheres, respectively. Both techniques are focus on the application of a strong electric field on a polymer solution, injecting a charge of a certain polarity onto the needle that dispenses and accelerates it towards the collector with opposite polarity. The property that characterizes these two types of structures is its porosity, which can change according to the parameters applied in its production [1].
Using the same setting, with simple modification of two parameters, structures with different properties are obtained. The combination of a polymer with a higher molecular weight and a weaker electric field will result in the assembly of nanofibers that will form a membrane. However, the use of a polymer with a lower molecular weight and the application of a higher electric field will lead to obtaining polymeric nanospheres.
The extensive study of these parameters allows us to exploit the high capacities of these systems with the aim of creating a versatile tool for optimizing catalytic and encapsulation processes, which can be used in applications such as drug delivery and therapy [2].

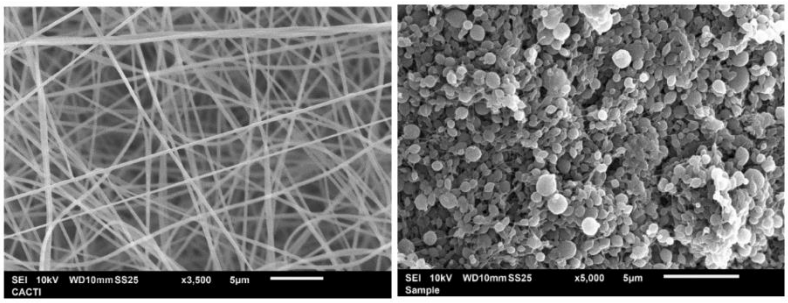

Figure 1. SEM images of nanofibers (left) and nanospheres (right) formed by polyvinyl alcohol (PVA).

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Development and characterization of the bacterial cellulose membrane impregnation process with 17-DMAG

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The compound 17-DMAG, a known anti-inflammatory agent initially tested as an anti-cancer therapy, may also hold promise for treating inflammatory diseases [1]. In cutaneous leishmaniasis, the localized delivery system offered by topical treatment is preferable, since a drug can be applied directly to the lesion site. Moreover, this approach is easy to administer and painless, minimizing the risk of side effects and systemic toxicity. Additionally, topical treatment avoids gastric degradation and hepatic metabolization [2]. Recently, bacterial cellulose membrane (BCM) has gained prominence as a nanomaterial in its gel form, due to biocompatibility, high water content, high absorption capacity, and protection against infection [3]. After pre-soaked membranes were cut to the desired size, excess water was removed, the drug amount (µg/cm²) was calculated, dissolved in water and applied to the membrane for an impregnation time of 6 hours. The membrane was then plated and dried in an oven at up to 30 ºC. However, following this protocol, we observed irregularities in drying time, with the membrane presenting a wrinkled appearance and sometimes adhering to the plate surface. After increasing surface area, a greater quantity of drug was consequently absorbed. To address these issues, we tested four conditions: leaving the membrane impregnated with water to dry in an oven (W+O) or in a lyophilizer (W+L), as well as using a 5% trehalose solution while drying in an oven (T+O) or in a lyophilizer (T+L). Analysis by scanning electron microscopy was performed to compare these processes. The trehalose-impregnated membrane presented a more homogeneous surface structure than those impregnated with water. While the T+O membrane presented a smooth appearance, drying times continued to be irregular. Although W+L impregnation also resulted in a smooth membrane, use trehalose was preferred due to its action as a cryoprotectant. We then evaluated the drug release profile of the membrane to assess absorption subsequent release. Membranes cut to a 3 mm diameter were treated at concentrations of 622, 311, and 155 µg/cm², then placed in an Eppendorf tube containing 1 mL of RPMI and incubated in an oven at 37 °C under rotational agitation. After 1, 3, 6, 24, 48, and 72 hours, the medium was collected and replaced. Quantification by high-performance liquid chromatography (HPLC) revealed, at every concentration tested, 100% release after 3 hours. In in vivo toxicity assays in which membranes were applied to dry murine ears, the drug was not released and membranes maintained a purple color, with no change in appearance. By contrast, when applied to wet ears, the membranes lost their purple color, indicating the release of 17-DMAG, and took on a thicker appearance. These results indicate that the adopted procedure is stable and reproducible, thus confirming the effectiveness of the impregnation process. Furthermore, it also shows that an aqueous medium is necessary for release to occur.

Acknowledgments: IGM technology platforms Funding: CAPES, CNPq, Fapesb, Fiocruz.

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Content of microplastics in stabilized sewage sludge

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Microplastics are estimated to be one of the biggest pollutants in the world. The annual production of plastics on a global scale is 300,000 tons, and only 10% of this is recycled [1]. In addition, most microplastics are difficult to degrade, which means that they will be in the ecosystem for hundreds to thousands of years [2]. Monitoring the amount of microplastics in the environment is both an important and challenging task. The qualitative and quantitative composition indicates the probable emission sources. An equally important task is to avoid the use of raw materials contaminated with microplastics, e.g. sewage sludge.
In the research, samples of stabilized sewage sludge were analyzed in terms of quantitative and qualitative analysis. The tested materials came from the wastewater treatment plant (WWTP) located in the south of Poland.The separation of microplastics was carried out in two stages. First, the sample matrix was digested with 15% hydrogen peroxide. The next step was density separation, where a saturated solution of calcium chloride was used. Separated microplastics were counted and their sources of origin were analyzed using a Raman confocal microscope and ATR FT-IR spectrometer. The samples were divided according to the month of their collection.
The June samples were characterized by the highest amount of microplastics - the average was about 2942 particles per 100 grams of dry weight of stabilized sewage sludge (Figure 1). The May samples had the lowest amount of microplastics, about 1745 particles per 100 grams of dry weight of stabilized sewage sludge. The dominant source of the identified microplastic is LDPE-Low Density Polyethylene. It is one of the most commonly used polymers. It is mainly used for the production of plastic bags.

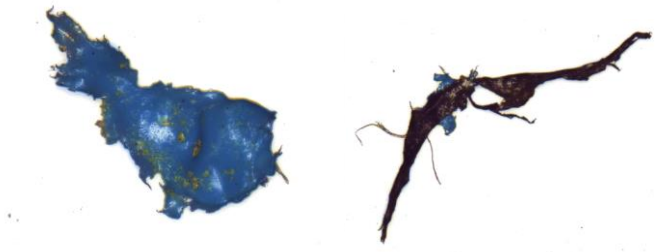

Figure 1. Separated microplastic from samples of stabilized sewage sludge.

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Chemical and bioactive evaluation of extracts produced from raspberry pruning material

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Due to its well-known high nutritional characteristics, raspberry production has expanded substantially in recent decades, and bioresidues arising from its cultivation have also increased [1]. Making highly valuable products out of these bioresidues, while also looking into their bioactivity potential, is one method for controlling the accumulation of these residues. The leaves and other aerial components of this raw material are an example of wasted biomass, which could be used in different industrial applications, such as cosmetics, due to their distinct chemical composition and associated functional properties [2]. Therefore, the aim of this study was to chemically characterize and explore the bioactive properties of different extracts obtained from *Rubus idaeus* L. aerial parts in an ecologically sustainable form.

Different extraction methods, namely infusion (Inf), maceration (MA), and decoction (Dec), as well as a more innovative extraction techniques, like ultrasound-assisted extraction (UAE), were explored and compared. The obtained extracts were characterized in terms of phenolic compounds by HPLC-DAD-ESI/MS. For the antioxidant activity evaluation, cell-based assays were tested, such as OxHLIA and TBARS. For the antibacterial activity, a microdilution technique against pathogenic bacteria was applied. The plant extracts' cytotoxic potential was determined using the sulforhodamine B technique, and their in vitro anti-inflammatory activity was assessed by testing their ability to reduce NO generation in RAW 264.7 cells.

Considering the phenolic profile, hydrolyzable tannins were the most abundant phenolic family, followed by ellagic acids and their derivatives, found in all extracts. The antioxidant activity results showed that raspberry samples had a comparable degree of activity, with extracts obtained by Dec revealing the most promising results, i.e., the lowest IC_{50} values in OxHLIA for a 60 min Δt, followed by MA. All the extracts showed similar antioxidant activity in comparison to trolox. On the other hand, extracts obtained by Inf and MA were more prominent in comparison with the other extracts, showing a higher inhibition in the TBARS formation. All the extracts had antibacterial activity, inhibiting the majority of the tested bacterial strains, thus, UAE showed a good effect against methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis*, with a minimum inhibitory concentration of 2.5 mg/mL. UAE and Inf extracts exhibited the lowest IC_{50} values regarding the anti-inflammatory activity, to which the UAE gave a better response with an IC_{50} of 27 μg/mL. This last extract also revealed the highest antitumor activity against all tested cell lines, presenting IC_{50} values of 93 μg/mL for the NCI-H460 cell line. Additionally, the results demonstrated that all of the extracts were innocuous against the PLP2 and VERO cell lines, showing no toxicity.

The combination of these results highlights the sample's bioactive potential and the significance of exploiting bioresidues as distinctive candidates for industrial applications.

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Fernando Cagide	**PC8-*TA***
Fernando H. Reboredo	**PC10-*HF***
Fernando M. Nunes	**OC1-*HF*, OC6-*HF*, PC19-HF, PC9-*TA***
Fernando M.H. Cardoso	**PC1-*SD*, PC8-*SD*, PC10-*HF***
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María Garranzo Asensio	**OC4-*SD***
Maria Inês Dias	**PC11-*TA***
María J. Blanco-Prieto	**PL8**
Maria J. Carmezim	**PC3-*TA***
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Patrícia D. Barata	**PC1-*SD*, PC8-*SD*, PC10-HF, PC17-*HF***
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S