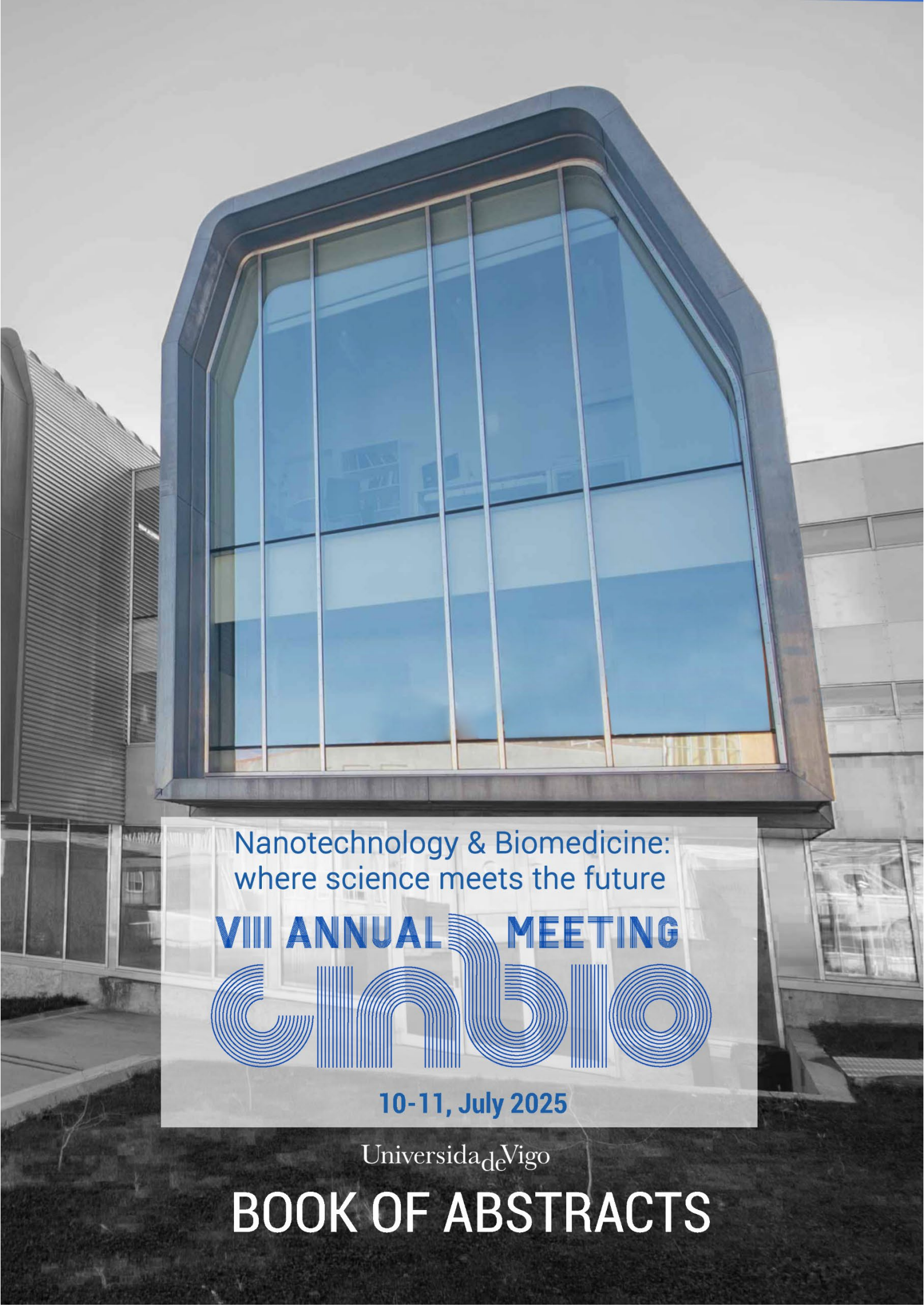

Nanotechnology & Biomedicine:
where science meets the future

VIII ANNUAL MEETING CINBIO

10-11, July 2025

Universidade Vigo

BOOK OF ABSTRACTS

10-11, July 2025

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VIII ANNUAL MEETING

CINBIO

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Prologue

We are delighted to present the proceedings of the 8th CINBIO ANNUAL MEETING, held in Vigo, Spain. This event aimed to bring together academic scientists and students to inspire, support, and celebrate exceptional scientific research.

Since its inception in 2017, the meeting has been entirely organized by CINBIO's postdoctoral researchers. It has evolved into a platform for interdisciplinary dialogue, fostering the exchange of experiences and research across a broad spectrum of scientific and social topics. Participants also addressed practical challenges, sharing insights into innovative solutions.

With representation from various countries, this year's meeting had a distinctly international character. Highlights included several oral presentations and two dynamic poster sessions that sparked engaging discussions and collaboration.

The program featured invited talks, technical workshops, and discussions led by distinguished experts, spanning an array of scientific disciplines. This diverse agenda offered participants abundant opportunities to network and exchange ideas.

We hope your participation in the 8th CINBIO Annual Meeting was both rewarding and impactful. Your involvement ensures the continued success of this conference, and we are deeply grateful for your contributions.

Our heartfelt thanks go to the organizing team, the scientific committee, as well as all speakers and poster presenters.

We look forward to welcoming you to the next edition, the 9th Annual Meeting in 2026, and eagerly anticipate your participation in making it another exceptional event.

Miguel Correa Duarte
Director of CINBIO

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VIII ANNUAL MEETING CINBIO

10th July

9:00 - 9:15 Registration
9:15 - 9:30 Opening and Welcome

SESSION 1

09:30 - 10:15 (KS1) Annemiek van Spriel Radboud Universiteit
Tetraspanins: molecular organisers of the immune cell surface

10:15 - 10:25 (ST1) Rocío Ferreiro Miguéns NasasBiotech S.L.
Microfluidic platform for EV-protein signature detection in lung cancer liquid biopsy
10:25 - 10:35 (ST2) Lara González Cabaleiro CINBIO, UVigo
Development of plasmonic nanoparticle-bacterial biohybrids for cancer therapy and biosensing
10:35 - 10:45 (ST3) Nair Varela Rouco CINBIO, UVigo
Mutational enrichment of circulating tumor cells in breast cancer
10:45 - 10:55 (ST4) Víctor Hernández Piñeiro Universidade de Coruña
Theoretical modelling of usage patterns for the life extension of Lithium batteries

11:00 - 12:00 Coffee & Poster session I

SESSION 2

12:00 - 12:30 (IS1) Jaime Martín Pérez Universidade de Coruña
Decoding the structure of donor polymer for organic solar cells

12:30 - 12:40 (ST5) Irune Fernández Rodríguez CINBIO, UVigo
Study of magnetically induced manipulation of microbeads in aqueous medium
12:40 - 12:50 (ST6) Qiuping Qian CINBIO, UVigo
In situ monitoring and protection of mitochondrial oxidative stress in living cells by SERS
12:50 - 13:00 (ST7) Diogo Moreira INL
A microfluidic platform for single-cell cancer biorecognition based on nanobodies

13:00 - 13:30 (IS2) Susana Carregal CIC biomaGUNE
Optimization of inhalable nanomedicines through the use of magnetic nanoparticles

13:30 - 15:00 Free Lunch Time

SESSION 3

15:00 - 15:30 (IS3) Alicia Torrado Lilly
DNA encoded library techonology in drug discovery

15:30 - 15:40 (ST8) Rita Fernandes de Matos INL
Development of a microfluidic device and EG-GFETs for the isolation, detection and analysis of circulating tumour DNA
15:40 - 15:50 (ST9) Iñaki López-Diaz CINBIO, UVigo
Extracellular vesicles from synovial fluid as biomarkers and imaging targets in osteoarthritis
15:50 - 16:00 (ST10) Andrés Serrano Freijeiro CINBIO, UVigo
Tunable plasmonic circular dichroism in reconfigurable chiral nanorod assemblies

16:00 - 16:30 (IS4) Verónica Montes ISIS, Université de Strasbourg & CNRS
Smart material-based sensors for selective and real-time chemical detection

16:30 - 16:40 (ST11) Patricia Alcázar Universidad de Oviedo & INL
Isothermal amplification for early detection of aquatic invasive species
16:40 - 16:50 (ST12) Aida Bilbao Lima CINBIO, UVigo
Evaluation of the role of CORO2B in ciliopathies
16:50 - 17:00 (ST13) José Nuno Gama CINBIO, UVigo
Light coupled by supramolecular structures: J-aggregate thin films supporting surface exciton polaritons

SESSION 4

17:00: MUSEUM VISIT

11th July

9:00 - 9:15 Arrival & Day 2 Welcome

SESSION 1

9:15 - 10:00 (KS2) Clèment Sanchez UPMC - Collège de France & CNRS
Integrative chemistry of functional nanostructured and hierarchically structured inorganic and hybrid materials

10:00 - 10:10 (ST14) Mafalda Neto INL, IBEC & UB
Unfolding organoids: reclaiming the three-dimensionality in intestinal organoid-derived monolayers
10:10 - 10:20 (ST15) Irene da Costa Barreiro CINBIO, UVigo
Indium Oxide-Supported Co-Fe Catalysts for the CO2-ODH of Ethane to Ethylene with Magnetic Induction Heating
10:20 - 10:30 (ST16) Mariana Costa INL
Impact of hemodynamic forces on brain endothelial and lung cancer cell interactions in brain metastasis

10:30 - 11:00 (IS5) Martina Huranova Institute of Molecular Genetics, Czech Academy of Sciences
Primary cilia in health and disease

11:00 - 12:00 Poster session II & Coffee

SESSION 2

12:00 - 12:30 (IS6) Rui Miguel Andrade Domingues INL
The cellular microenvironment and submolecular control of their mechanosignaling in tendon tissue specification and function

12:30 - 12:40 (ST17) Tania María Sampayo Roca INIBIC, Universidade da Coruña
Bioinkable hydrogels for cartilage regeneration: physicochemical and functional evaluation
12:40 - 12:50 (ST18) Sara Rodríguez Da Silva CINBIO, UVigo
Precipitation synthesis of size-controlled chiral MOF
12:50- 13:00 (ST19) Hugo Silva INL
Leveraging deterministic lateral displacement in menstrual blood analysis
13:00 - 13:10 (ST20) Manuel Fernández Míguez CiQUS, USC
Synergistic communication mechanism: double step sergeants and soldiers effect

13:10 - 13:40 ROUNDTABLE: *Advices about the research career for young new PhDs: things not to be forgotten*
María Gallardo - IISGS & IPO
Saheen Pathan - USC & CINBIO, UVigo
Ana Covelo - CINBIO, UVigo
Nicolás Ramos - UVigo

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(KS) Keynote speaker (IS) Invited speaker (ST) Short Talk

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POSTER SESSION II

Talks

Tetraspanins: molecular organisers of the immune cell surface

Annemiek B. van Spriel[1]

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The plasma membrane of cells contains thousands of different proteins including receptors, enzymes and membrane-bound signalling molecules. A tight spatiotemporal organisation of these membrane proteins is essential for cell function and the result of a dynamic exchange between protein monomers, nanoscale protein clusters, and microscale higher-order structures. The superfamily of four-transmembrane tetraspanin proteins play a central role in organising membrane proteins and signalling molecules into specialized nanodomains[1].

The aim of our research is to understand the molecular mechanisms underlying tetraspanin function in the plasma membrane of immune cells and their malignant counterparts. I will present our latest findings into the function of tetraspanins CD37 and CD20 in lymphocytes and B cell lymphoma. We report that CD37 inhibits activation of the IL-6 signalling pathway at the cell surface of B cells, and CD37-deficiency initiates the development of B cell lymphoma. Glycosylation of the extracellular domain of tetraspanins affects their capacity to interact with other membrane proteins. In addition, cell surface proteomics revealed novel interaction partners that are important for cell-cell interactions. Together, our data demonstrates that tetraspanins are essential for immune cell function, which provides opportunities to develop therapeutic approaches that act via the modulation of membrane organization.

References:

[1] Querol Cano L, Dunlock VE, Schwerdtfeger F, van Spriel AB. Membrane organization by tetraspanins and galectins shapes lymphocyte function. *Nat Rev Immunol*. 2024 Mar;24(3):193-212

Microfluidic Platform for EV-Protein Signature Detection in Lung Cancer Liquid Biopsy

Ferreiro, Rocío[1]; Lage, Teresa[2], Macedo, Daniela[2]; Bravo, Susana[3]; Abal, Miguel[4]; García, Jorge[4,5]; Honrado, Carlos[2]; Diéguez, Lorena[2]; León, Luis[4,5]

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Introduction: Liquid biopsy (LB) analyzes tumor-derived material in biofluids, including CTCs, ctDNA, and extracellular vesicles (EVs). EVs are stable nanovesicles (40–160 nm) carrying protected cargo (DNA, RNA, proteins, lipids), making them promising non-invasive cancer biomarkers. Lung cancer is the leading cause of cancer mortality, often diagnosed late due to non-specific symptoms. Effective, non-invasive screening tools are urgently needed. In this sense, microfluidics, already applied in the isolation and analysis of CTCs [1], offers new opportunities to improve the capture and detection of EVs for clinical applications [2].

Objective: To develop an EV-based proteomic signature using ExoGAG [3] to differentiate between lung cancer patients and healthy individuals, and to functionalise a microfluidic chip to improve the isolation and detection of EVs

Methodology: EVs were isolated from plasma (n=46; 30 NSCLC, 16 controls) using ExoGAG. Proteins were analyzed by SWATH-MS, with bioinformatics analysis via STRING and R. EVs were characterized by NTA. In parallel, a microfluidic chip is being developed for selective EV capture and improved detection of the signature.

Results: A total of 683 proteins were identified, 180 differentially expressed (92 upregulated, 88 downregulated in patients), involved in transcriptional and tumor-related pathways. A biomarker panel was defined and evaluated individually and as a combined EV signature. Integration into the chip is underway to assess diagnostic performance.

Conclusions: An EV-based proteomic signature for lung cancer was identified. A microfluidic approach is under development to enhance its detection, supporting early diagnosis and future clinical application.

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Development of plasmonic nanoparticle-bacterial biohybrids for cancer therapy and biosensing

González-Cabaleiro, Lara[1,2,3,*], Vázquez-Iglesias, Lorena[1,2], Bodelón, Gustavo[1,4], Pérez-Juste, Jorge[1,2,3], and Pastoriza-Santos, Isabel[1,2,3]

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Bacterial cells have emerged as a promising tool in cancer therapy due to their natural ability to target tumors, high motility, immunogenic properties, and capacity for on-demand therapeutic agent expression. The integration of living bacterial cells with inorganic components has led to the development of functional biohybrids, expanding material applications in unprecedented ways at the intersection of biology and materials science. Among these components, plasmonic nanoparticles stand out for their potential in nanomedicine, excelling in drug delivery, photothermal and magnetothermal therapies, photodynamic therapy, and imaging via surface-enhanced Raman scattering (SERS). As a result, combining bacterial cells with nanomaterials to create biohybrid microrobots offers a powerful, multifaceted approach to cancer treatment.[1]

This study aims to establish robust protein-protein interactions to facilitate the assembly of the nanoparticles (NPs) on bacterial surfaces, creating a flexible and efficient platform with anticancer potential. We report the successful development of bacterial-NP hybrids using Spytag/SpyCatcher covalent binding, demonstrating their application as live biohybrid microrobots that merge plasmonic nanoparticles with genetically engineered bacteria to serve as a novel anticancer tool.[2,3] To achieve this, gold nanorods (AuNRs) and gold hollow nanocapsules functionalized with SpyCatcher were orthogonally assembled onto *Escherichia coli* cells expressing SpyTag fused to the intimin display system. The targeting and bioimaging capabilities of the reprogrammed bacterial biohybrid bearing synthetic adhesins against the human epidermal growth factor receptor (EGFR) tumor biomarker were evaluated in vitro using hollow codified nanocapsules for SERS, and the treatment capabilities of the AuNRs were tested by photothermal therapy (PTT) at 1064 nm laser line. Initial results confirmed the successful functionalization of the nanoparticles with SpyCatcher. The resulting NP-SpyCatcher-*E. coli* expressing Spytag complex demonstrated specific binding to cultured cells expressing EGFR, and following bioimaging (SERS) and treatment (PTT).

References:

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Mutational enrichment of circulating tumor cells in breast cancer

Varela-Rouco, Nair[1,2,*], Estévez-Gómez, Nuria[1,2], Fernández-Santiago, Cristóbal[3], Tomás, Laura[1,2], Alves, João Miguel[1,2], Piñeiro, Roberto[3], Posada, David[1,2]

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Circulating tumor cells (CTCs) play a crucial role in cancer metastasis and are associated with poorer clinical outcomes. CTC clusters represent aggregates of tumor cells traveling together in the bloodstream, exhibiting increased metastatic potential compared to individual CTCs due to their enhanced survival, collective migration capabilities, and resistance to apoptosis and shear stress. However, the genomic heterogeneity within these clusters and their relationship with primary tumors and metastases are not well understood. To explore this, we conducted whole–exome sequencing on single CTCs, CTC clusters, primary tumors, and metastatic samples from a triple–negative breast cancer cell line–derived mouse xenograft. Our findings revealed that while single CTCs and CTC clusters displayed marked genomic similarity, these circulating cell populations showed extensive genomic divergence from primary tumors and/or metastases. Importantly, we identified multiple genes with non–silent mutations more frequently in CTCs and CTC clusters than in primary tumors and/or metastases (Figure 1), suggesting that these genes might play critical roles in the formation, survival, and metastatic potential of CTCs and CTC clusters. This study provides novel insights into the genomic landscape of circulating tumor cells and pinpoints genes of interest that might represent potential therapeutic targets for preventing cancer dissemination.

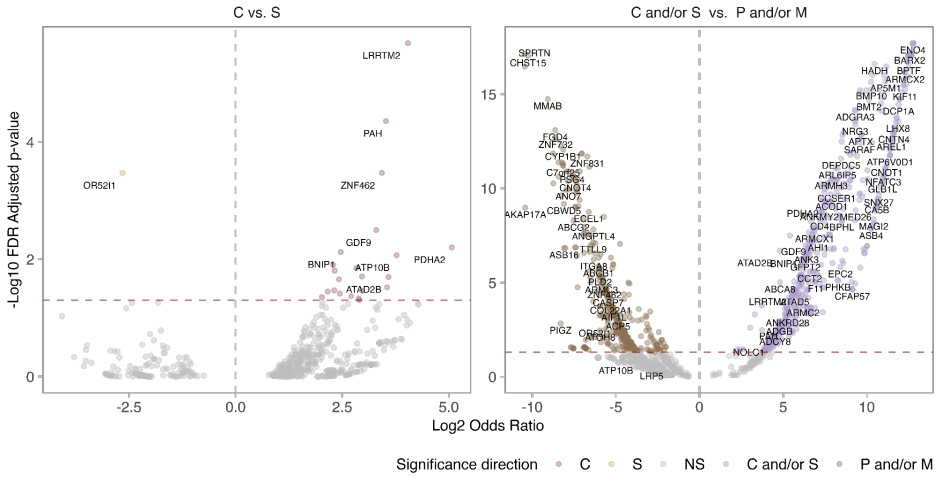

Figure 1. Pairwise mutational enrichment analysis. Comparisons of genes with non–silent SNVs between CTC clusters (C) and single CTCs (S), and between those two *versus* primary tumors (P) and/or metastatic samples (M). The dashed grey vertical line marks $\log_2$ OR = 0 (no enrichment), and the dashed red horizontal line marks the FDR–adjusted p–value threshold of 0.05 (–log10 0.05). Significant gene names are labeled. SNVs, single–nucleotide variants; OR, odds ratio; FDR, false discovery rate.

This work has been funded by the grant PID2019-106247GB-I00 awarded to D.P. and by the PhD fellowship PRE2020-092269 to N.V-R, both from the Spanish Ministry of Science and Innovation (MICINN).

Theoretical modelling of usage patterns for the life extension of Lithium batteries

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One of the major challenges of our time is the development of clean and sustainable energy. Renewable sources play a crucial role in this transition, but their intermittency poses a significant obstacle: efficient energy storage.[1] Among the various storage technologies, lithium-ion batteries stand out as a leading solution due to their high energy density and versatility. However, improving battery performance and extending their lifespan remains an active area of research. A key limitation in this field lies in the time and resources required to conduct battery cycling tests, which are essential for evaluating long-term behaviour. These tests can take several months, significantly slowing down development and innovation.

To address this bottleneck, alternative strategies are being explored, with machine learning emerging as a powerful tool. By leveraging data from previously tested and cycled batteries, it is possible to train models capable of predicting key performance indicators, such as the long-term state of health [2]. This work focuses on the theoretical modelling of lithium-ion batteries using machine learning, more specifically using Random Forest, with a specific aim: predicting the optimal slurry composition for cathodes rich in nickel. The study evaluates how different parameters—such as the percentage of active material, carbon black, and other compounds—affect battery performance. Rather than directly extending battery lifespan, this work seeks to understand the relationships between these parameters and identify which ones can lead to improved performance.

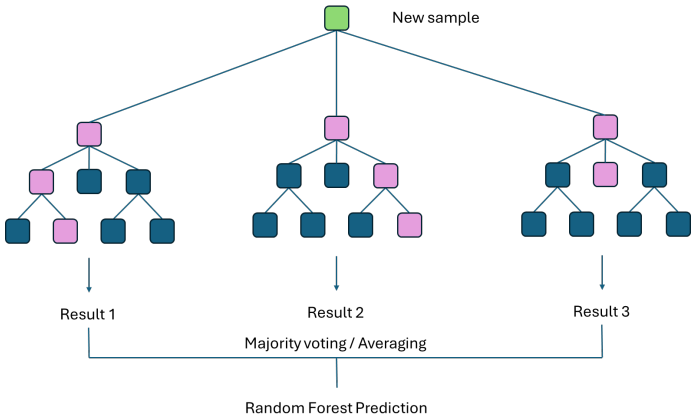

Figure 1. Random Forest Scheme

References:

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Decoding the Structure of Donor Polymers for Organic Solar Cells

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The performance of organic electronic devices, such as solar cells, depends on understanding and controlling the solid-state microstructure of semiconducting polymers. In this talk, I will discuss on our recent understanding of the aggregate states, solid-state microstructure, and thermotropic behavior of the best-performing family of polymers for solar cells, i.e., benzodithiophene-based semiconducting polymers. I will argue that the microstructure of these polymers does not seem to fit neither in the traditional structural models developed for polymers, i.e. the amorphous and semi-crystalline models, nor with other stablished low-order structures, a.k.a. solid mesophases, such as condis crystals, liquid crystals, hexagonal phases, columnar phases, paracrystals, and so on. Conversely, these polymers organize into a unique solid mesophase comprising stacked solid-like and liquid-like layers. This mesophase resembles sanidic structures while also sharing features with columnar mesophases like condis-crystals and paracrystals. At a larger length-scale, the mesophase organizes into nanoscale fibril-like domains, with polymer backbones aligned along the fibril axis, coexisting with amorphous-like glassy regions, reported in our work for the first time. I will also discuss on the thermotropic behavior of this biphasic nanomorphology, providing insights into how thermal annealing influences polymer structure. The understanding of the structure of semiconducting polymers enables a more precise framework for defining structure-function relationships, impact thus on the entire field of organic electronics, from organic photovoltaics to bioelectronics to wearable electronics

Study of magnetically induced manipulation of microbeads in aqueous medium

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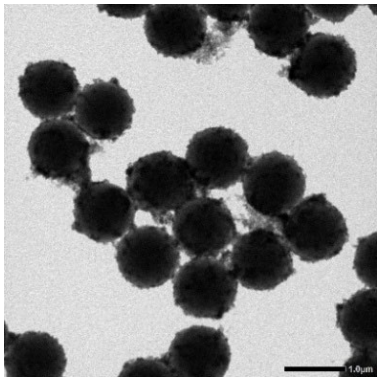
The induced and controlled movement of magnetic microbeads in suspension, both in aqueous solutions and more complex fluids, is gaining significant interest due to its potential in bio-related applications.

To explore this potential, we synthesized magnetic microbeads comprising a polystyrene spherical core with diameters of 500 nm and 1000 nm. These cores were coated with one, two, and three layers of magnetite nanoparticles. The resulting nano- and microstructures were thoroughly characterized using a combination of techniques, including dynamic light scattering (DLS), scanning and transmission electron microscopy (SEM and TEM), Raman spectroscopy, X-ray diffraction (XRD), and vibrating sample magnetometry (VSM).

Subsequently, these magnetic microbeads were employed in controlled experiments designed to study the factors influencing their displacement. The investigations considered both internal factors, such as the structural architecture of the microbeads, and external stimuli, specifically magnetic field gradients.

The motion of these submicron- and micron-sized beads was analyzed using an experimental setup that incorporated a magnetic field generator (MFG-100i) connected to an inverted optical microscope. This configuration enabled real-time visualization and video acquisition of the bead movement. To differentiate between purely diffusive motion and motion induced by external magnetic fields, optical tracking was conducted to extract bead trajectories. The mean squared displacement (MSD) was computed to quantitatively assess the dynamics of the microbeads under varying experimental conditions [1], [2].

(A)



(B)

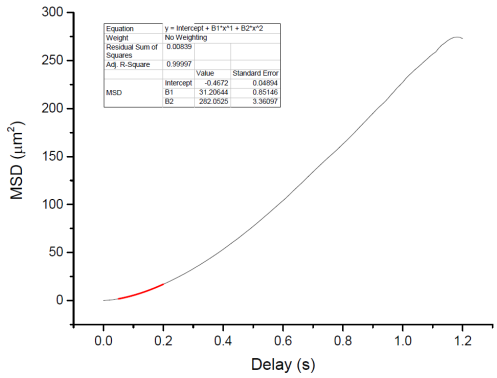


Figure 1. (A) Representative TEM image of the magnetic microbeads employed and (B) graph showing MSD vs short periods of time.

References:

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In situ monitoring and protection of mitochondrial oxidative stress in living cells by SERS

Qiuping, Qian[1], Carlos Renero-Lecuna[2],Gustavo Bodelón Gonzalez[3,*], Luis M. Liz-Marzán [4,*]

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The excessive accumulation of ROS may induce oxidative stress and subsequent mitochondrial dysfunction Mitochondrial dysfunction contributes to diseases such as neurodegeneration, cancer, and metabolic syndromes. [1,2] Therefore, enhancing mitochondrial function is essential for maintaining normal cellular homeostasis; at the same time, label-free monitoring of mitochondrial status is critically important. We report a rough-structured gold nanoparticle SERS probe for in situ and non-invasive monitoring of mitochondrial oxidative stress by SERS. Enhanced localized surface plasmon resonance at nanoparticle hot spots amplifies Raman signals, boosting sensitivity and spatial resolution. The gold nanoparticles were functionalized with TPP-PEG-SH and DSPE-PEG-SH for mitochondrial targeting and sensing, respectively. Furthermore, a functional mitochondria-targeting peptide (CRRRRRRRRRYKF) was also co-assembled on the peptide–polyphenol nanoparticle. Upon Cu^{2+} or H_2O_2 exposure, the SERS-based probe monitors membrane potential changes, and cytochrome c changes. Raman signatures under different stimuli revealed unique oxidative pathways (be more specific). Include a statement indicating the assessment of the nanomaterial´s biocompatibility. As shown, the nanoprobes achieved mitochondrial targeting, in situ H_2O_2 scavenging , and enabled SERS-based, real-time monitoring of mitochondrial oxidative stress, paving the way for a new tool that can provide new molecular insights for early diagnosis and targeted therapies of mitochondrial diseases.

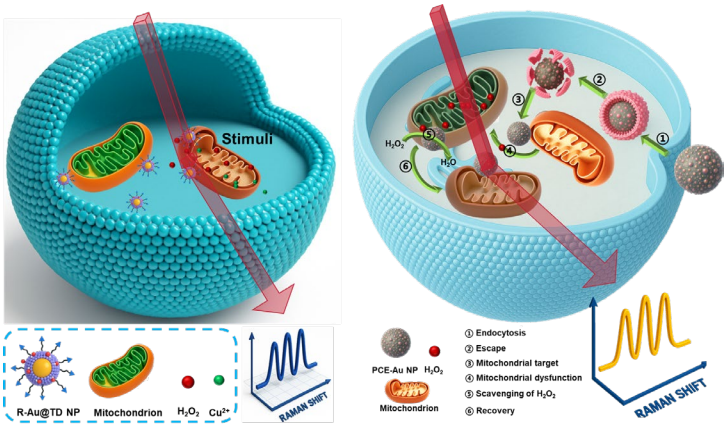

Figure 1. In situ monitoring of mitochondria by R-Au@TD NP (Left, rough-surfaced gold nanoparticles were functionalized with HS–PEG–TPP and SH–PEG–DSPE to create nanoprobes, hereafter abbreviated as R-Au@TD NP), In situ protection and monitoring of mitochondria in living cells by PCE-Au NP (Right, peptide–cysteine–epigallocatechin gallate co-assembled with chloroauric acid to form gold nanoparticles, abbreviated as PEC-Au NP).

Acknowledgements

We acknowledge funding from the European Commission through Grant No.101130615 and Grant No. ERC-2024-SyG 101166855.

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A microfluidic platform for Single-Cell Cancer Biorecognition based on nanobodies

Moreira, Diogo[1,*], Teixeira, Alexandra[1], Sánchez, Eva[2], Rodrigues, Carolina[1], Fraga, Joana[3], Chícharo, Alexandre[4], Honrado, Carlos[1], Diéguez, Lorena[1,3], Fernández, Luis Ángel [2], Abalde-Cela, Sara[1,3]

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Breast cancer is one of the most diagnosed cancers worldwide, affecting primarily women. Female breast cancer accounts for 11.6% of all new cancer diagnoses [1]. Current methods are not very efficient in the early detection of metastases; therefore, more sensitive methods are needed for the detection. Circulating tumour cells (CTCs) serve as biomarkers for cancer monitoring and prognosis. However, when these CTCs are isolated, single-cell analysis remains challenging due to technological limitations, especially given the scarcity of CTCs requiring sophisticated isolation methods and high cost for high throughput technologies [2,3,4]. A new lab-on-a-chip system combining microfluidics, synthetic biology and surface-enhanced Raman Scattering (SERS) spectroscopy is being developed to analyse these CTCs by leveraging microdroplets technology single-cell encapsulation. The phenotypic characterization of the CTCs is achieved by co-encapsulation of the CTCs with bioengineered bacteria (*E. Coli*), through nanobodies expressed in the membranes of the later. In this study, co-encapsulation (1:300) of different strains of bacteria and different model cancer cell lines (SKBR3, MDA-MB-231, MDA-MB-453, BT-474) was achieved successfully in 2D, 3D and in within a microfluidic isolation chip for CTCs. The expression of these nanobodies on the bacteria membrane allowed the recognition of specific surface markers (EGFR, Her2, PDL1, TIR) present in the membrane of the CTCs. Further, bacteria recognition was demonstrated by co-encapsulating bacteria and cultured cancer cells in microdroplets and the fluorescence emitted by the bacteria allowed the observation of their clustering around the cell membrane.

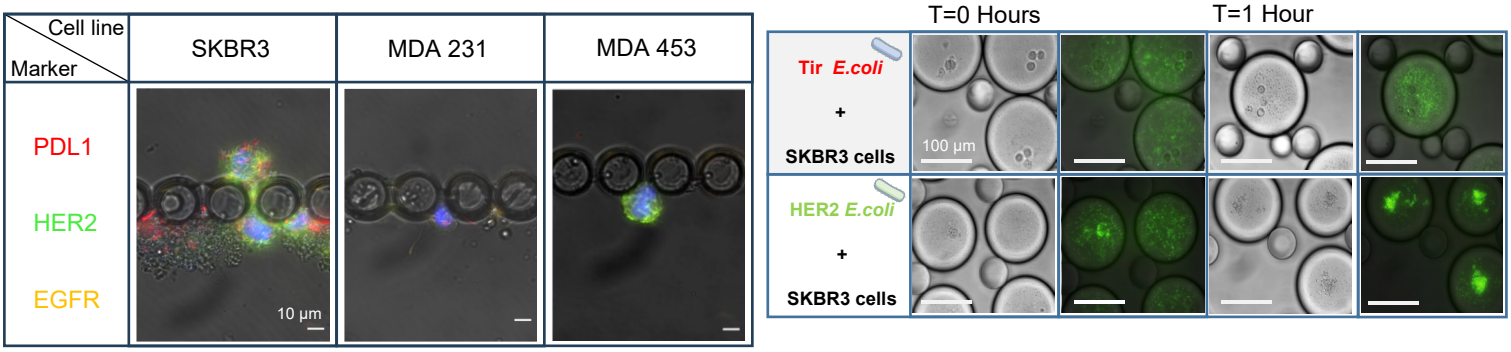

Figure 1- Multiplexing labelling with different breast cancer cell lines models (left) and labelling of SKBR3 cell lines over time with a positive and negative control.

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Optimization of inhalable nanomedicines through the use of magnetic nanoparticles

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Pulmonary administration of nanomedicines, including viral vaccines, offers a direct, noninvasive route for both systemic delivery and lung-targeted therapy. However, natural lung defense mechanisms often lead to rapid clearance or degradation, limiting clinical translation. This challenge contrasts with the growing burden of lung diseases in aging populations and emerging infectious threats, underscoring the need for optimized pulmonary nanomedicines.

We employ bottom-up synthetic protocols to develop iron oxide nanoparticles for magnetic resonance imaging (MRI) or bimodal positron emission tomography (PET)/MRI imaging. These nanoparticles are encapsulated or integrated into lipid- or protein-based drug carriers. Using proteomics and molecular imaging, we analyze their interactions with lung barriers.

Our findings show how molecular imaging provides insights into lung retention, penetration, and cellular uptake. Moreover, we highlight the use of magnetic separation in studying lung surfactant corona formation. We present multifunctional nanoparticles capable of drug encapsulation and contrast agent labeling, enabling multi-scale characterization of nano-bio interactions. Finally, we show how different surface coatings influence lung retention, macrophage clearance, and tissue penetration, impacting nanomedicine efficacy.

DNA Encoded Library (DEL) Technology in Drug Discovery

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DNA-encoded library (DEL) technology is a powerful platform for small-molecule discovery that has gained prominence in both academia and the pharmaceutical industry over the past 20 years. This approach involves linking chemical building blocks to oligonucleotides through DNA-compatible synthesis, allowing for the efficient creation and screening of large and diverse libraries. DEL technology offers several advantages, including minimal resource requirements, easy multiplexing, and streamlined screening processes, making it ideal for discovering bioactive ligands for various therapeutic targets. The success of DELs relies on the availability and diversity of chemical building blocks, robust DNA-compatible reactions, and thoughtful library design.

Development of a microfluidic device and EG-GFETs for the isolation, detection and analysis of circulating tumour DNA

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Cancer is one of the leading causes of death worldwide. Early diagnosis using fast and non-invasive methods is urgent, since early detection can reduce mortality and increase chances of survival [1]. Electrolyte-gated graphene field effect transistors (EG-GFETs) have emerged as promising biosensors, particularly for DNA mutation detection, due to their distinguished properties such as high sensitivity, selectivity, and fast response time [2]. However, most studies reporting EG-GFETs fail to address the challenge of working with complex fluids. In blood, the noise originating from non-targets reduces selectivity and limits sensitivity, hampering result interpretation [3]. Thus, prior isolation or enrichment of the target is beneficial. This would be particularly important for early-stage cancer detection, where ctDNA levels are very low. Potentially, enrichment can be achieved using microfluidic-based technologies [4]. However, research on ctDNA isolation using microfluidics is typically followed by traditional DNA analyses, which are poorly suited for point-of-care. Here, we propose a miniaturised platform for early-stage cancer detection by combining microfluidics and EG-GFETs to extract and detect ctDNA (Fig.1).

EG-GFETs were fabricated by photolithography (Fig.1A). The electrodes (Cr/Au) were sputtered, while the single layer graphene channels were grown via thermal chemical vapor deposition (Fig.1B). Each sensor was integrated with a bespoke PCB designed to interface with a custom-made measuring device. Our early data shows the expected V-shaped curve demonstrating the successful production of EG-GFETs (Fig.1C). For microfluidic enrichment, we initially replicated the METRO protocol [5], but fabricating a silicon master mould via deep-reactive ion etching for PDMS replica moulding. Preliminary experiments demonstrated that surface treatment with Pluronic F-127 effectively prevented DNA adsorption to PDMS surfaces, validating its use for DNA extraction. Future tests will explore the use of a fluidised bed containing magnetic beads with charge-switchable amine-terminated groups for DNA capture.

This dual-modality approach lays the groundwork for a sensitive, miniaturised, and clinically relevant platform capable of detecting trace levels of ctDNA at the point of care.

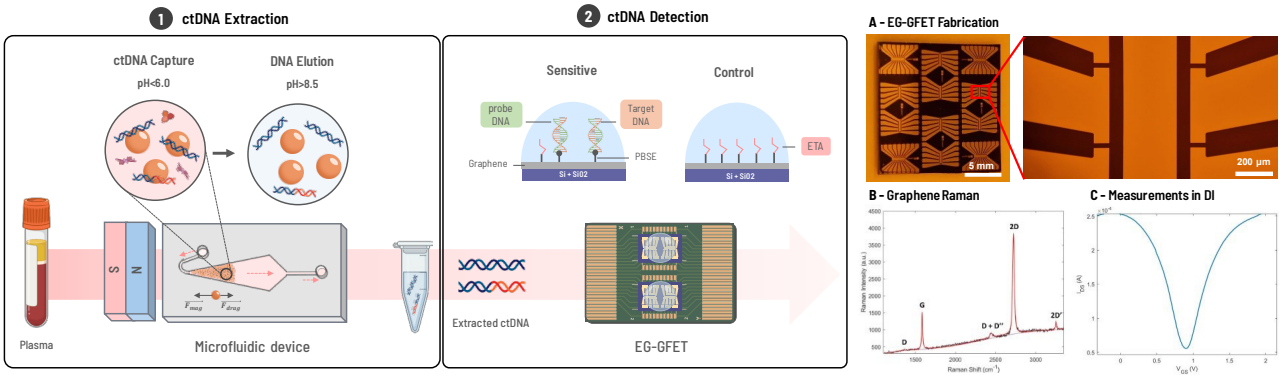

Figure 1. General workflow of the microfluidic and EG-GFET ctDNA assay and main results of the EG-GFETs.

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Extracellular Vesicles from Synovial Fluid as Biomarkers and Imaging Targets in Osteoarthritis

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Osteoarthritis (OA) is a degenerative joint disease and the most common form of arthritis. It is one of the leading causes of pain and disability worldwide. It is characterised by joint pain, limited mobility and an impaired quality of life, primarily due to the deterioration of articular cartilage and the subsequent loss of joint function. These changes are often accompanied by synovial inflammation, osteophyte formation and subchondral bone remodelling. Our research group has identified the transmembrane protein connexin43 (Cx43) as a key contributor to OA progression, from early stages through to later stages of the disease, due to its role in promoting cellular senescence and inflammation. We have also discovered that Cx43 present in extracellular vesicles (Cx43-EVs), which may be released into the synovial fluid, could serve as a promising biomarker for early diagnosis. With this in mind, our aim was to optimise the isolation of extracellular vesicles (EVs) from synovial fluid using animal samples prior to transitioning to patient-derived samples. Cx43 was detected in the EVs isolated from synovial fluid samples for patients and in higher concentrations regarding a non-OA pathology. Next, the isolated EVs were isolated by density gradient ultracentrifugation and analysed using flow cytometry to identify and quantify the various subpopulations present in the samples based on different dyes and markers and in order to identify the subpopulations positive for Cx43. These subpopulations were sorted using flow cytometry-based cell sorting. In parallel, EV size and concentration were measured via nanoparticle tracking analysis (NTA). Also, the use of advanced microscopy techniques allowed us to visualize the isolated EVs providing crucial insights into their morphology, size distribution and structural characteristics. By integrating these complementary techniques into a unified workflow, our aim is to establish standardized methodologies for detecting Cx43-positive EVs in synovial fluid, to explore their use as biomarkers for early disease diagnosis and to expand knowledge about their role within these vesicles—ensuring reproducibility.

EVEREST Consortium, funded under Marie Skłodowska-Curie Actions (MSCA); 101183034

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Tunable Plasmonic Circular Dichroism in Reconfigurable Chiral Nanorod Assemblies

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Plasmonic circular dichroism (PCD) is a powerful approach to enhance chiroptical effects at the nanoscale, enabling applications in chiral sensing, enantioselective catalysis, optical information processing, and dynamic photonics. While most strategies rely on either intrinsically chiral nanoparticles (NPs) or achiral NPs arranged into chiral assemblies, combining both offers a promising route to amplify optical asymmetry. In this work, we employ depletion-induced self-assembly (DISA)[1-2] to organize fourfold-twisted chiral gold nanorods (cGNRs)[3] into hierarchical chiral superstructures with reconfigurable PCD properties. By reducing cGNR helicity via chemical etching, we study how building block chirality influences suprastructure formation. Evaporation-induced self-assembly (EISA) on substrates yields static helical supercrystals, whereas DISA in solution allows dynamic and reversible tuning of interparticle distances (7–13 nm) by varying surfactant concentration. Our approach enables rapid assembly/disassembly cycles with g-factor variations of two orders of magnitude (from 0.0016 to 0.2 at 740 nm), confirmed by SAXS, TEM, and CD spectroscopy. The PCD response was found to be highest in compact, side-by-side assemblies formed at intermediate CTAC concentrations (90–200 mM), with the system showing full reversibility over multiple cycles. This ligand-exchange-free strategy is compatible with various NP shapes, offering a scalable platform for designing isotropic, reconfigurable chiral nanofluids. Though response times remain too slow for optical computing, these findings are relevant for tunable electro-optic modulators and memory devices based on active chiral nanomaterials.

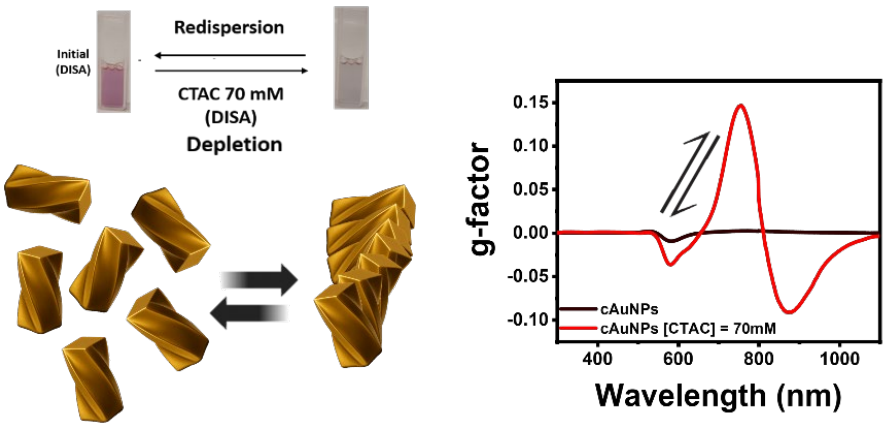

Figure 1. Scheme and optical properties of chiral gold nanoparticles assembly reversibility.

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Smart Material-Based Sensors for Selective and Real-Time Chemical Detection

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The development of next-generation chemical sensors hinges on the ability to achieve high selectivity, sensitivity, and real-time operation in complex environments. In this talk, I will present three complementary approaches that leverage smart materials and molecular engineering to address these challenges. First, I will discuss a supramolecular chemiresistor platform based on 3D gold nanoparticle networks for selective potassium ion detection in biofluids, enabling real-time monitoring relevant for point-of-care diagnostics.1 Next, I will highlight a novel strategy for humidity sensing using covalently functionalized MXenes, which offers a broad operating range, fast response time, and exceptional selectivity in the presence of volatile interferents.2 Finally, I will introduce an integrated sensing approach combining surface-enhanced Raman scattering (SERS) with machine learning algorithms for the ultrasensitive and robust discrimination of structural, geometric, and optical isomers.3 Together, these examples illustrate how the rational design of nanostructured materials and the integration of advanced data analysis can push the boundaries of chemical sensing across biomedical and environmental domains.

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Isothermal Amplification for Early Detection of Aquatic Invasive Species

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Aquatic invasive species (AIS) represent a serious threat to ecosystems worldwide, affecting local diversity and potentially leading to the extinction of native species. Early detection is crucial to take effective measures to control their spread [1].

This project works with catfish (*Silurus glanis*), an invasive species in several rivers of the Iberian Peninsula –including the Guadalquivir. An optical method based on gold nanoparticles (AuNPs) is employed for detecting this species. The methodology involves changes in the aggregation state of AuNPs, leading to alterations in their maximum absorption, which results in a colorimetric signal. To enhance sensitivity, AuNPs are functionalized with nucleic acid enzymes known as Multicomponent Nucleic Acid Enzymes (MNAzymes), enabling an isothermal signal amplification assay [2]. For the detection of catfish, a specific DNA sequence has been designed, achieving detection in the picomolar range.

Current studies at the International Iberian Nanotechnology Laboratory (INL) are being focused on optimizing DNA purification and concentration methods from water samples. A microfluidic device incorporating chitosan-based micropillars is being developed to efficiently capture and elute catfish DNA [3]. We expect that combining these technologies will provide a rapid, sensitive, and non-invasive approach to monitoring aquatic ecosystems, enabling management actions to prevent the establishment and spread of invasive species.

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Evaluation of the role of *CORO2B* in ciliopathies.

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Bardet-Biedl Syndrome (BBS) is a ciliopathy caused by disfunction of cilia, evolutionarily conserved structures present in nearly all vertebrate cells that act as signalling hubs for receiving and transmitting extracellular signals. Mutations in the *CORO2B* gene, which encodes a protein of the coronin family, have been identified in individuals with BBS. Coronins are actin cytoskeleton regulatory proteins involved in the assembly of multiprotein complexes, and their malfunction has been linked to various diseases [1, 2].

This study aims to characterize the role of two CORO2B mutations (p.Leu194Gln and p.Pro318Leu) identified in BBS patients. To investigate the protein's function, we conducted CORO2B modification and labelling assays in two cell lines, U-87 MG and HEK, to assess its role in each model.

Immunoprecipitation assays were performed to evaluate interactions between CORO2B and other cellular components, with a focus on actin filaments and vinculin. qPCR and western blot analyses compared expression levels of mutant and wild-type (WT) CORO2B. Immunofluorescence assays were used to analyse colocalization of CORO2B (WT and mutants) with actin filaments. Additionally, apoptosis assays were performed to assess cell viability in mutant-expressing cells and under conditions of absent CORO2B expression.

Significant differences were observed across all assays, suggesting that CORO2B mutations may contribute to the pathogenesis of BBS and potentially other ciliopathies.

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LIGHT COUPLED BY SUPRAMOLECULAR STRUCTURES: J-AGGREGATE THIN FILMS SUPPORTING SURFACE EXCITON POLARITONS

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The field of polaritonics has seen significant advancements in the past decades due to the utilization of plasmonic materials like gold and silver [1] in areas such as biosensing, energy and information technologies [2]. Yet, these raw materials usually come with critical sustainability issues.

In this work, we bring an alternative by venturing into the realm of organic polaritonics by investigating surface exciton polaritons (SEPs) in molecular materials at room temperature. This alternative is based on carbocyanines with optical response ranging from the visible to the telecom wavelengths [3]. These molecules have electronic properties that make them ideal for self-assembling into supramolecular structures that promote in-lattice delocalization of Frenkel excitons which bring them with strong sharp excitonic resonances [4]. The presence of these strong resonances is responsible for reaching negative values in the real part of the permittivity, supporting SEPs at the material's interface. However, the control of conformation of supramolecular structures for the growth of solid films with optical quality is a challenging task. We solved this problem by following a Layer-by-Layer (LbL) assembly [5] on flat substrates. First, due to unique electronic properties of cyanine monomers, we were able to stabilize and control the dispersity of the J-aggregates by tuning the chemical properties of water as a solvent and stirring over a large period, respectively. Later, we deposited the self-assembled J-aggregates by dipping alternately a functionalized substrate in the J-aggregate solution and in an aqueous polymer with positive charge – poly(diallyldimethylammonium chloride) (PDDA). Taking advantage of the negatively charged functional groups of cyanines, their corresponding J-aggregates were consistently absorbed subsequently with the polyelectrolyte into the surface of the film, creating multi-layers.

Using this approach, we fabricated molecular films with narrow-band optical properties from five different J-aggregates with spectral ranges varying from 560 nm to 1000 nm with accurate control over their thickness. Then, using spectroscopic ellipsometry we estimated each of their dielectric functions, seeking for negative values in the real part. Finally, we corroborated the SEP coupling, after successfully exciting and detecting the confined SEP mode living at the interface between the J-aggregate films and the air medium [6], using advanced Fourier imaging spectroscopy combined with a typical Kretschman prism-coupling configuration.

Our work paves a new way for the creation of tailored Vis-NIR polaritonic materials with the potential for nano-structuring controlling distances between different species. Such advancements will enable the exploration of more sophisticated light-matter interaction mechanisms, opening doors for innovative polaritonic devices functionalities.

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Integrative chemistry of functional nanostructured and hierarchically structured Inorganic and hybrid materials

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Hybrid inorganic-organic materials can be broadly defined as synthetic materials with organic and inorganic components which are intimately mixed. They can be either homogeneous systems derived from monomers and miscible organic and inorganic components, or heterogeneous and phase-separated systems where at least one of the components' domains has a dimension ranging from a few Å to several nanometers. Hybrid phases can also be used to nanostructure or texture new inorganic nanomaterials (porous or non-porous). The versatile synthetic conditions provided by bottom-up strategies such as reactive molecular precursors or clusters, tunable processing temperatures and solvents and the adjustable rheology of the colloidal state allow for the mixing of the organic and inorganic components at the nanometer scale in virtually any ratio. These features, and the advancement of organometallic chemistry and polymer and sol-gel processing, make possible a high degree of control over both composition and structure (including nanostructure) of these materials, which present tunable structure-property relationships. This, in turn, makes it possible to tailor and fine-tune properties (mechanical, optical, electronic, thermal, chemical…) in very broad ranges, and to design specific systems for applications. Hybrid materials can be processed as gels, monoliths, thin films, fibers, particles or powders or can be intermediates to design materials having complex shapes or hierarchical structures. The seemingly unlimited variety, unique structure-property control, and the compositional and shaping flexibility give these materials a high potential in sensing, membranes, catalysis, biocatalysis, photocatalysis, nanomedicine, the tailoring of smart functional surfaces etc…. This lecture will describe some recent advances on this integrative materials chemistry that allows via a chemistry-process coupling to tailor made nanostructured and hierarchically structured functional inorganic and hybrid materials. Some of their properties will also be discussed.

Unfolding organoids: reclaiming the three-dimensionality in intestinal organoid-derived monolayers

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Organoids provide superior physiological relevance than traditional *in vitro* models due to their cellular diversity and self-organisation, yet their closed architecture restricts apical access, limiting functional studies. [1] Although organoid-derived monolayers established using 2D culture systems enable direct access to the apical surface, they continue to fall short in matching the cell renewal and differentiation capacity intrinsic to 3D organoids. [2] To address this, we designed an organ-on-chip device integrating spatiotemporal environmental control and a tissue-specific decellularised extracellular matrix (dECM), emulating the *in vivo* milieu for organoid-derived culture. Organoids were generated from human colonic crypts and cultured in Matrigel. [3] Prior to differentiation, intestinal organoids displayed cystic morphology and a polarised epithelial barrier. Differentiation for 7 days induced budding structures and expression of markers for specialised cells, including goblet (MUC2) and enteroendocrine cells (chromogranin A). To establish monolayers, expanded organoids were dissociated and seeded on culture inserts for 21 days. They similarly exhibited secretory cells by day 7, maintained a polarised barrier with a dense apical brush border, and expressed intercellular junctions (ZO-1) as shown in Figure 1. Transepithelial electrical resistance increased until day 14 (Fig 1b), indicating enhanced barrier integrity. The apparent permeability to Lucifer Yellow was closer to values found *ex vivo* compared to immortalised cell co-cultures (Fig 1c). As expected, both differentiated 3D organoids and their derived monolayers showed decreased stem cell marker expression and increased differentiation markers, except for Lysozyme, a marker for Paneth cells, which reside in the stem cell niche. While both showed similar loss of stem cells, 3D organoids had a stronger increase in differentiation markers, particularly Chromogranin A. These findings demonstrate robust formation and characterisation of 2D monolayers from 3D organoids, while still evidencing their limitation in recapitulating the full complexity of 3D organoids. Ongoing work involves decellularising intestinal tissue to develop the tissue-specific hydrogel for culturing these monolayers under dynamic conditions in an organ-on-chip with a central chamber accommodating the dECM.

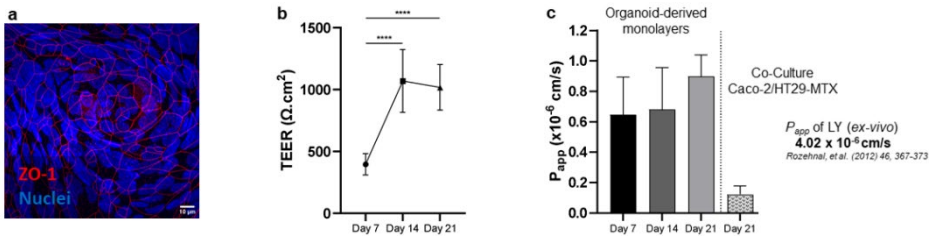

Figure 1. Organoid-derived monolayers exhibit effective barrier function.

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Indium Oxide-Supported Co-Fe Catalysts for the CO2-ODH of Ethane to Ethylene with Magnetic Induction Heating

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Oxidative dehydrogenation of ethane using CO_2 as a soft oxidant (CO_2-ODH) offers a sustainable alternative to the conventional energy- and capital-intensive ethylene production routes, enabling ethane-to-ethylene conversion under milder conditions while reducing anthropogenic CO_2 emissions. Despite its potential, selective activation of C–H and C–O bonds at high temperatures remains a challenge. Magnetic induction heating, proven effective in methane dry reforming [1], shows promise for advancing CO_2-ODH and related catalytic processes.

To address these challenges, efficient magnetic catalysts were developed by incorporating Fe and Co onto In_2O_3 nanoparticles, with Co:Fe molar ratios of 1:3, 1:1, 3:1 at a 5 wt.% metal loading [2]. A comprehensive set of advanced characterization techniques-including *operando* XAS and XRD at synchrotron facilities, H_2-TPR, CO_2-TPD, Raman spectroscopy, VSM, STEM-EDS, and TEM/SAED-was used to elucidate the structure, magnetism, and catalytic performance, providing insights into the active site during the reaction process.

Catalytic tests conducted under magnetic induction heating identified the material with a Co:Fe ratio of 1:3 as the best-performer (Figure 1A). For this ratio, XAS and TEM/SAED verified the predominant formation of cobalt ferrite ($CoFe_2O_4$, Figure 1B), which generates heat through magnetic induction and is epitaxially grown on In_2O_3, collectively serving as the active phase. The materials with higher Fe or Co contents exhibited additional secondary oxides, such as Fe_2O_3 and CoO, which affected catalyst performance. These non-magnetic oxides reduced stability, while Fe_2O_3 improved ethylene selectivity but lowered ethane conversion, and CoO increased ethane conversion at the cost of reduced selectivity.

These results demonstrate the potential of cobalt-iron bimetallic catalysts for sustainable ethylene production *via* CO_2-ODH and highlight magnetic induction heating as innovation in catalytic processes.

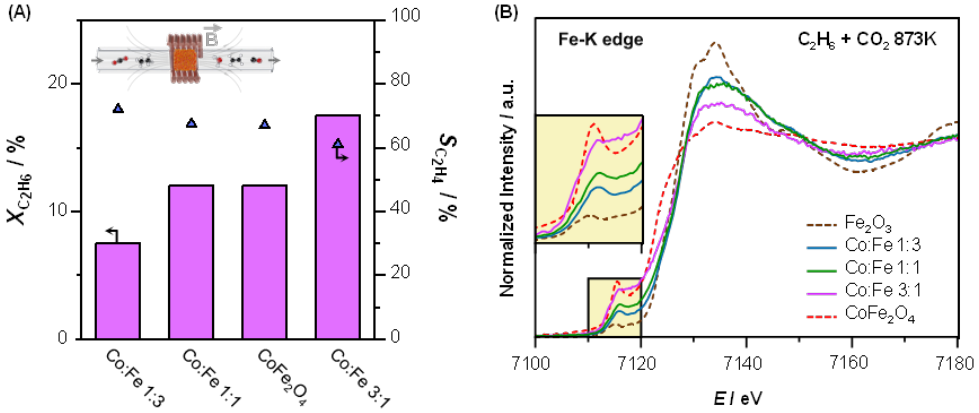

Figure 1. (A) Catalytic activity of various catalysts under magnetic induction. (B) Fe-K edge XANES spectra of catalysts during reaction.

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Impact of Hemodynamic Forces on Brain Endothelial and Lung Cancer Cell Interactions in Brain Metastasis

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Cancer metastases account for 90% of cancer-related deaths [1], showing the devastating impact of metastasis on patients and highlighting the importance of studying its mechanisms. In lung cancer, metastasis occurs through the dissemination of circulating tumour cells (CTCs), which mostly target the brain (15% to 43%) [2]. Once CTCs reach the blood-brain barrier (BBB), they undergo a process of extravasation to escape circulation [3]. However, this process is not yet fully understood, owing to the structural and physiological function of the BBB together with the complexity of in vivo systems. Although challenging, the development of new dynamic and biomimetic models to study lung CTC extravasation to the brain is crucial for evaluating the specific impact of hemodynamic forces during CTC extravasation. Microfluidic systems are promising platforms to mimic physiological microenvironments under controlled conditions [4]. The development of a microfluidic model of brain metastasis facilitates the study of how hemodynamic forces influence the extravasation of lung CTCs into brain parenchyma, one of the key processes within the metastatic cascade. In this work, we propose to fabricate a single-channel PDMS-based microfluidic device with main dimensions of 400 µm height, 16 mm length and 0.25 cm2 of working area functionalised with ECM proteins, specifically collagen type I and fibronectin. An endothelial cell monolayer was established under low shear stress conditions (2x10-5 Pa) to ensure consistent nutrient supply. Ongoing assays are focused on evaluating endothelial cell response to various levels of shear stress through Live/Dead assay, growth kinetics and immunocytochemistry for expression of membrane proteins, and assessment of cell-cell interactions between endothelial and lung cancer cells. Therefore, my master's thesis work consists of developing a microfluidic device to explore the interaction between brain endothelial cells and lung CTCs under distinct hemodynamic forces. Overall, we believe this study can contribute significantly to advancing personalised medicine approaches for cancertreatment and prevention.

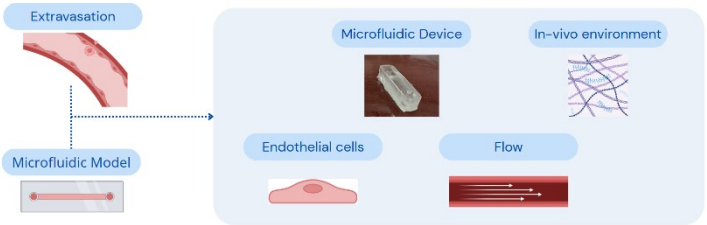

Figure 1. Components of the Blood-Brain Barrier (BBB) model

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Primary Cilia in Health and Disease

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Primary cilia are microtubule-based structures extending from the surface of most mammalian cells, excluding hematopoietic cells. Once thought to be vestigial, cilia are now recognized as vital organelles involved in signal transduction and sensing extracellular cues. Cilia house receptors for key signaling pathways like Sonic Hedgehog (SHH) and WNT, essential for tissue development and function. Genetic mutations disrupting cilia function lead to ciliopathies, a group of disorders with diverse symptoms, including obesity, renal cysts, retinal degeneration, polydactyly, and neurological issues. Our research focuses on understanding cilia's physiological roles and the mechanisms behind ciliopathies, with a particular emphasis on Bardet-Biedl Syndrome. We investigate ciliary dynamics and receptor turnover in developmental signaling, aiming to identify potential therapeutic approaches for these disorders.

The cellular microenvironment and submolecular control of their mechanosignaling in tendon tissue specification and function

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Tendons exhibit complex physiology and poor regenerative capacity, making the treatment of tendinopathy a significant challenge. Tendon's heterogeneous cell population is composed of distinct subtypes with specialized functions, within a dynamic extracellular matrix (ECM) with context-dependent composition and architecture. As highly mechanosentive tissues, resident cells are primed by different types if mechanical forces transduced by specialized cell membrane mechanoreceptors. However, the biological mechanisms driving the onset and progression of tendinopathy remain poorly understood. A major limitation in the field is the lack of representative human in vitro models that can provide mechanistic insights into tendon homeostasis, development, and disease, which are often difficult to study in vivo using animal models. But beyond the tendon microenvironment, we also currently lack experimental tools enabling us to unveil the mechanotransduction mechanisms mediated by specific cell force sensors such as PIEZO channels.

To address this gap, we have applied a range of bioengineering approaches to develop 3D human-based microphysiological systems. This talk will focus on the design principles and fabrication strategies we have explored to develop these tendon-on-chip models using two distinct platforms: conventional microfluidic devices and automated 3D writing within support matrices. While differing in technical implementation, both approaches share the common goal of recreating the anisotropic architecture, cell organization, and phenotype of the intrinsic (core) tendon compartment and its bidirectional crosstalk with different tendon cell populations of the extrinsic compartment, including vascular system and adaptive immune cell responses. This talk will address how we are developing a new technology exploring molecular imprinting concepts combined with magnetic systems to produce tailor-made nanoswitches enabling targeted wireless actuation on PIEZO channels with submolecular precision in different cells relevant for tendon physiology, with potential for regenerative applications.

Overall, the merging of these two technologies will provide powerful new tools for investigating the biological mechanisms and signaling pathways underlying tendon physiology and pathology, as well as for evaluating potential therapeutic strategies.

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Bioinkable hydrogels for cartilage regeneration: physicochemical and functional evaluation

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Osteoarthritis (OA) is a disease that affects the joints, causing cartilage degradation and cellular stress. Current treatments do not stop its progression or restore cartilage, highlighting the need for new regenerative therapies. This study evaluates hydrogels with optimal properties for bioprinting applications in OA.

Two hydrogels composed of gellan gum, konjac, and alginate were used: Hydrogel A (without collagen) and Hydrogel B (with collagen). Their diffusion and swelling behavior were assessed using crystal violet, cytotoxicity was evaluated in human keratinocytes (ISO 10993-12:2021), and the cell viability of mesenchymal stem cells (MSCs) was quantified by live/dead cell immunofluorescence. Additionally, the chondrogenic differentiation of MSCs into chondrocytes over 21 days was analyzed, along with the expression of genes associated with chondrogenesis, such as the transcription factor SRY-Box9 (SOX9), collagen type II (COLII), and proliferating cell nuclear antigen (PCNA).

Results showed that Hydrogel A exhibited greater swelling and diffusion than Hydrogel B, although it also underwent more degradation. Both hydrogels rapidly absorbed the dye, with Hydrogel A showing higher diffusion, likely due to its lower component concentration. Both materials demonstrated cell viability above 70% (ISO 10993-5), with Hydrogel B exceeding 100% viability compared to the control, suggesting that collagen supports cell proliferation. Immunofluorescence revealed cell clustering and a slight increase in cell death over time. Despite seeding challenges, cell distribution within the hydrogels was uniform. Finally, significant expression of SOX9 and COLII was detected compared to undifferentiated MSCs, indicating potential chondrogenic differentiation within the hydrogel. The PCNA marker showed significantly lower levels in differentiated cells, supporting this differentiation process.

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Precipitation Synthesis of Size-Controlled Chiral MOF.

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The **Metal-organic frameworks** (MOFs), composed of metal ions or clusters coordinated to organic linkers, are highly porous crystalline materials known for their exceptional properties—including high target affinity, selectivity, and molecular sieving—which make them attractive for a wide range of applications.[1] Among them, chiral MOFs are gaining increasing attention due to their potential in enantioselective catalysis, chiral separation, and sensing. However, achieving uniform chiral MOF crystals with controlled size and shape remains a major synthetic challenge..

The **room temperature precipitation method** involves mixing metal and organic precursors under ambient conditions to induce supersaturation, triggering rapid nucleation and subsequent crystal formation. This approach often leads to a broad particle size distribution due to the competing kinetics of mixing, nucleation, growth and secondary processes such as agglomeration, aggregation and disaggregation.[2] Therefore, fine-tuning these parameters is critical to achieving monodisperse and well-defined crystals.

In this study, we present the synthesis and characterization of a new chiral MOF (TAMOF-1, Figure 1), consisting of Cu2+ as metal ion, which forms hexacoordinate complexes with the organic linker (S)-3-(1H-Imidazol-5-yl)-2-(4H-1,2,4-triazol-4-yl) propanoic Acid (HTA). The synthesis is based on a room-temperature precipitation strategy that enables controlled nucleation and crystal growth, yielding uniform and homogeneous particles. By modulating synthesis parameters such as reaction time and stirring conditions, we demonstrate precise control over particle size, paving the way for the optimization of TAMOF-1 in future chiral applications.

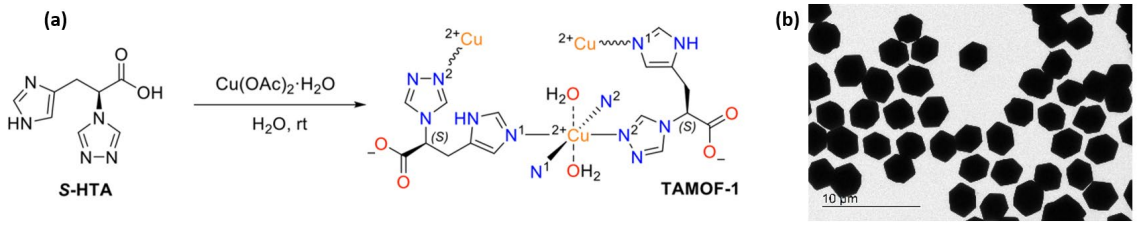

Figure 1. *(a) TAMOF synthesis (b) Representative TEM image of TAMOF nanocrystals.*

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Leveraging Deterministic Lateral Displacement in Menstrual Blood Analysis

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The World Health Organization (WHO) acknowledges the significant impact of endometriosis (ED) on sexual and reproductive health, well-being, and quality of life, while also calling it "the last health taboo" underscoring the gravity of a condition long shrouded in stigma and misinformation. ED affects around 190 million women and individuals assigned female at birth, and it is a chronic, inflammatory, hormone-dependent condition marked by tissue similar to the uterine lining growing outside the uterus. It increases risks of infertility, chronic fatigue, pain, and other comorbidities, severely disrupting daily life, relationships, and work. Many patients experience debilitating symptoms and face long diagnostic delays—averaging 7.5 years from symptom onset [1]. Therefore, raising awareness, correcting misconceptions, and improving diagnosis and treatment are essential. Recent advances in diagnoses have identified specific DNA Single Nucleotide Polymorphisms (SNPs) that could enable early diagnosis of ED. As such detection requires access to cell DNA, tissue samples are still the gold-standard, however they are invasive and impractical in clinical settings. Alternatively, we aim to leverage the presence of nucleated cells within menstrual fluid (MF) to obtain the necessary DNA material [2]. Our goal is thus to isolate such cells from the complex MF taking advantage of their larger size versus other MF components (e.g. red blood cells, platelets). Microfluidic deterministic lateral displacement (DLD), is a promising approach for precise separation and sorting of cells. DLD functions by guiding particles through an array of regularly spaced obstacles within a microfluidic channel. Smaller particles follow the fluid flow (zig-zag motion), while larger particles (such as nucleated cells) are laterally displaced due to interactions with the structured pillars. By modifying pillar size, shape, and spacing, fine-tuning of the threshold size for separation is achieved [3]. This study aims to optimize a DLD array, with cut-off size of 7 µm (**Figure 1**), for isolating nucleated cells from menstrual fluid through three steps: benchmarking with polystyrene beads (preliminary data shows sorting efficiency of >95% for 10 µm particles); proof-of-concept testing with PBS spiked with peripheral blood mononuclear cells (PBMCs); and validation using lysed whole blood. Integrating this method with Point-of-Care diagnostics offers a promising, non-invasive solution for early endometriosis detection, potentially reducing diagnostic delays and advancing menstrual health awareness.

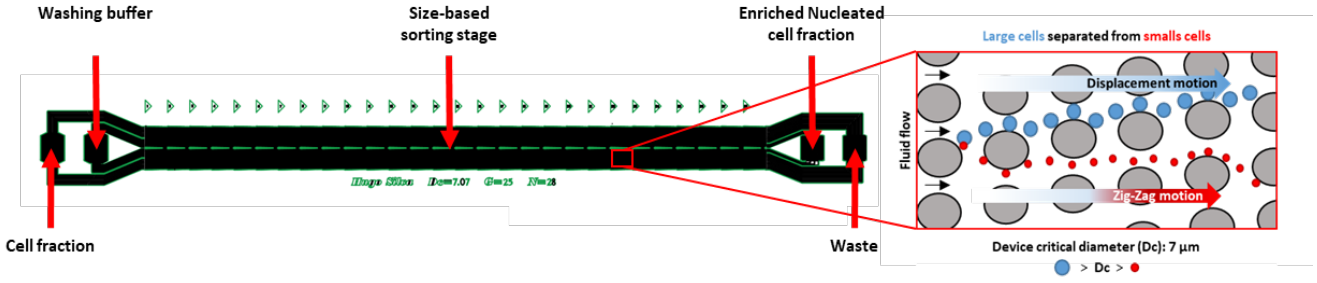

Figure 2: Schematic depiction of the developed DLD device and sorting mechanism

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Synergistic Communication Mechanism: Double Step Sergeants and Soldiers Effect

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Sergeants and Soldiers Effect (SaS) is classically found in copolymers composed of Achiral Monomers that act as Soldiers, and Chiral Monomers that act as Sergeants.1,2 This assumption exists for chiral molecules that induce chirality into the helix. In this project, we want to study the communication mechanisms in poly(phenylacetylene)s (PPAs) random copolymers where the chiral monomers induce axially racemic structure in the homopolymers, as do the achiral monomers. For the copolymerization, chiral monomer (S)-1 (para-substituted) and achiral monomers 2 (para-substituted) or 3 (meta-substituted) were synthesized, characterized, and copolymerized to form poly-[(S/R)-1r-co-2/31-r].3 In polar solvents, the copolymers showed a classical SaS, where the chiral monomer (S)-1 acts as a classical Sergeant. While in apolar solvent like CHCl3, despite both (S/R)-1 and 2/3 forming axially racemic homopolymers, the hydrophobic environment from 2 or 3 induced helicity in (S/R)-1. This initial activation combined with a subsequent SaS effect results in a new cascade SaS mechanism in the para-substituted achiral monomer 2. UV-Vis and ECD spectra confirmed that elongation and chiral structures depend only on the monomer ratio, obtaining compressed copolymers with chiral induction.

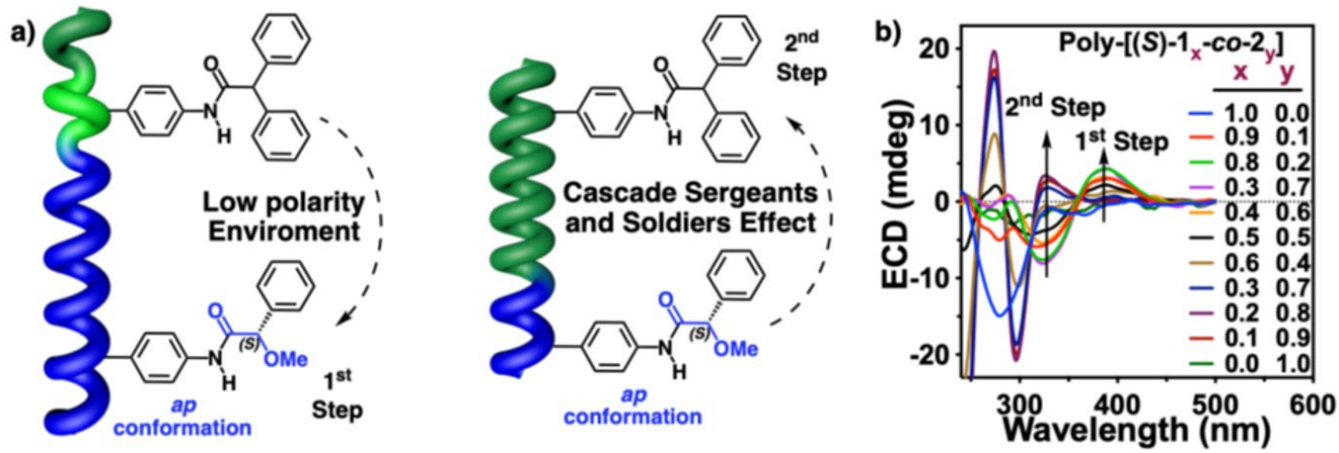

*Figure 1. a) Schematic Representation of the Double step communication Mechanism in poly-[(S/R)-**1**$_r$-co-**2**$_{1-r}$]. b) ECD spectra of poly-[(S/R)-**1**$_r$-co-**2**$_{1-r}$].*

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POSTER SESSION

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Electrospun Membranes Embedding Plasmonic–Semiconductor Hybrids for Enhanced Ammonia Photocatalysis

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The combination of plasmonic nanoparticles (PNPs) with semiconductor photocatalysts represents a promising approach to the development of high-performance photocatalysts. These hybrid nanoparticle configurations have demonstrated enhanced catalytic efficiency compared to plasmonic or semiconductor catalysts when used separately. However, the complex recovery process and lack of long-term colloidal stability present significant challenges to the widespread application of these hybrid nanoparticles in catalysis. To address these limitations, hybrid nanoparticles have been integrated into polymer fibers, resulting in the creation of functional photocatalytic membranes. [1].

In this work, hybrid photocatalysts composed of TiO_2 nanoparticles and gold nanostars (AuNSts), were supported on SiO_2 spheres, which were subsequently added into a polyvinyl alcohol (PVA) solution to create a hybrid electrospun membrane. The resulting hybrid membrane was used in a continuous-flow photocatalytic system to produce ammonia (NH3) from N2 using sunlight.

The results showed that the SiO_2@AuNSts@TiO_2 hybrid produced 2033 µmol/g*h of NH3, significantly more than the hybrids containing only AuNSts or TiO2, highlighting a 15-fold increase over the use of TiO_2 alone under experimental conditions of 2 mL/h. The hybrid membrane exhibited a continuous production over 96 h, demonstrating the efficiency and stability of the membrane in the continuous-flow photocatalytic system.

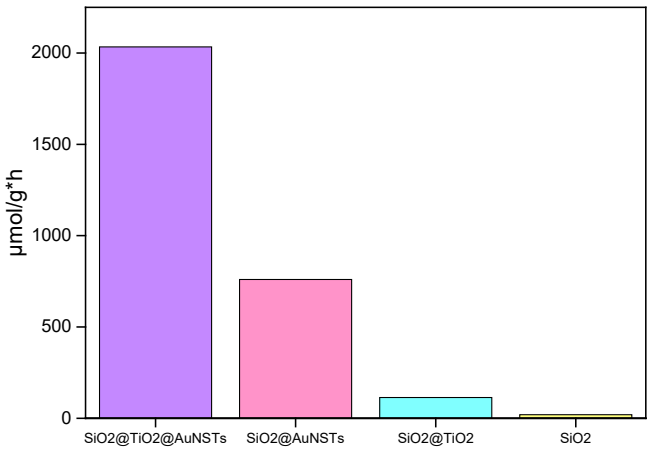

Figure 1. Graph showing the absorbance of samples of different hybrids to produce ammonia in a continuous photocatalysis system.

Acknowledgements: Spanish Ministerio de Ciencia e Innovación (project TED2021-130038A-I00/AEI/10.13039/501100011033).

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Biopolymer-Based Nanocarriers to Tackle Antimicrobial Resistance

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Polymeric nanoparticles have gained considerable attention as versatile drug delivery systems, offering the capacity to modulate pharmacokinetic and pharmacodynamic profiles while enabling sustained and site-specific release of therapeutic agents. In particular, their application in host-directed therapies provides a promising strategy to combat antimicrobial resistance (AMR). This study focuses on the development of nanoparticles based on polycaprolactone (PCL), a biodegradable and bioresorbable polymer known for its mechanical strength and controlled degradation, and alginate (ALG), a biocompatible natural polysaccharide valued for its low toxicity and functional versatility in biomedical applications. Nanoparticles composed of PCL and PCL-ALG blends were synthesized using two different fabrication techniques: a conventional method and electrospray processing. A comparative evaluation was conducted to determine the advantages and limitations of each approach.

Entinostat was employed as a model compound due to its reported immunomodulatory properties, which may contribute to enhancing host immune defenses against AMR pathogens. The drug release profile was studied under simulated physiological conditions, including simulated gastric (SGF) and intestinal fluids (SIF), providing insights into the nanoparticles' potential for achieving controlled and targeted drug delivery.

Overall, this work demonstrates the adaptability of PCL and PCL–ALG nanoparticles and highlights their potential as promising nanocarriers for the design of next-generation therapeutic platforms aimed at addressing the growing challenge of antimicrobial resistance.

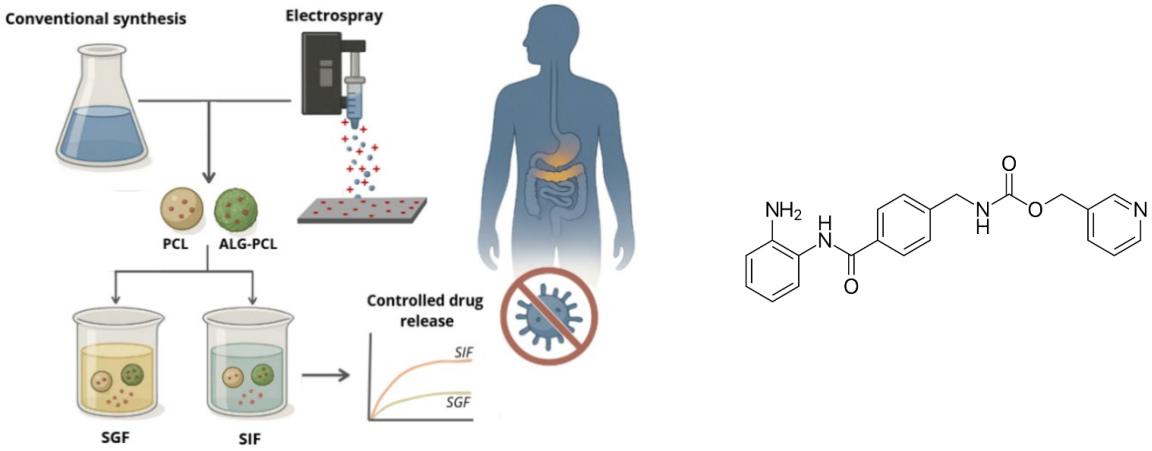

Figure 1. Synthesis and gastrointestinal drug release of polymeric nanoparticles (left) and Entinostat molecule (right).

Acknowledgement of Funding: IN-ARMOR, HORIZON-HLTH-2022-DISEASE-06-two-stage.

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Electrospray-Coated Mesoporous Silica Nanoparticles for Efficient and Scalable Drug Delivery Systems

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Mesoporous silica nanoparticles (MSNs) are promising platforms for oral drug delivery due to their large surface area, tunable pore structure, and ability to encapsulate therapeutic agents for controlled, pH-responsive release [1,2]. To overcome limitations of conventional surface modification methods, often slow and poorly scalable, we employed a novel coaxial electrospray technique to functionalize MSNs with chitosan@alginate and lipidic coatings [4]. Chitosan and alginate layers were deposited via electrostatic interactions [5], while lipidic coatings were formed through liposome fusion onto MSN surfaces [6]. Electrospray parameters were optimized to ensure coating homogeneity and particle integrity. Characterization by TEM, DLS, zeta potential, and FTIR confirmed successful core–shell structure formation and coating-specific chemical signatures [5,6]. Drug release assays in simulated gastric and intestinal fluids revealed pronounced pH-responsive behavior, with electrospray-coated MSNs exhibiting improved release control and structural stability compared to conventionally coated counterparts. These results highlight electrospray as a scalable and tunable method for MSN functionalization, enhancing their potential for advanced oral drug delivery applications.

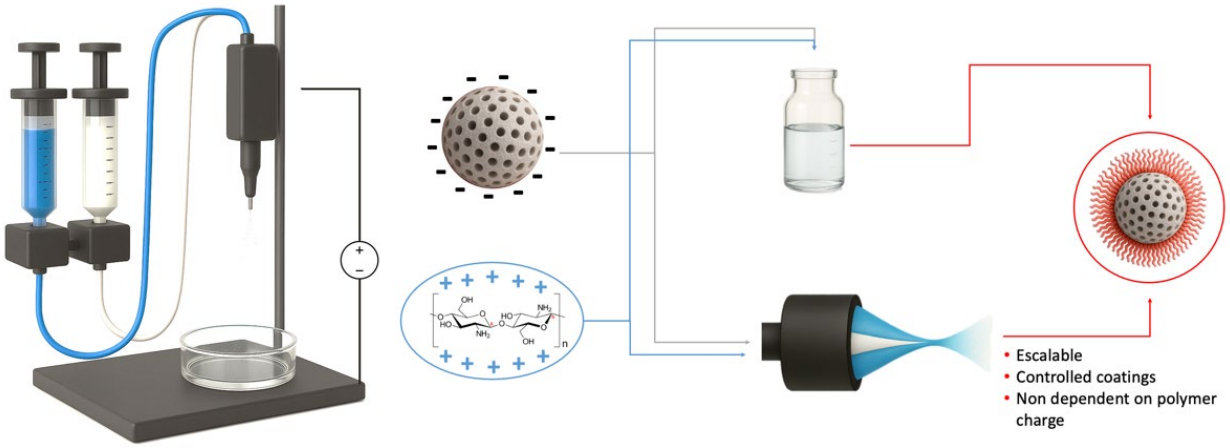

Figure 1. Coaxial electrospray rendering (left); overview of proposal (right).

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Morphology Control in 2D COFs via Systematic Reaction Parameter Adjustment

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Two-dimensional covalent organic frameworks (2D COFs) are highly tunable, crystalline, and porous materials with immense potential in areas such as gas storage, catalysis, and electronics [1]. However, achieving precise control over their morphology and particle size remains a challenge. Therefore, understanding how these are influenced by the synthetic conditions is essential for developing reproducible and scalable production methods.

In this study, we investigate the effects of reaction parameters such as solvent choice, reaction temperature, and time, on the morphology and crystallinity, of the COF synthesized from 1,3,5-tris(4-aminophenyl)benzene (TAPB) and 2,5-dimethoxyterephthalaldehyde (DMTP; hereafter referred to as TAPB-DMTP) under solvothermal conditions. A systematic variation of these parameters was conducted to elucidate their influence on the morphology (Figure 1).

Morphological analysis was performed using field-emission scanning electron microscopy (FESEM), while crystallinity was assessed by powder X-ray diffraction. Specific surface area and porosity were determined via N_2 physisorption. The results highlight how subtle changes in reaction conditions can significantly impact the COF morphology and yield. This study offers valuable insights for optimizing synthesis protocols, enabling better control over material quality and more efficient production of high-performance 2D COFs.

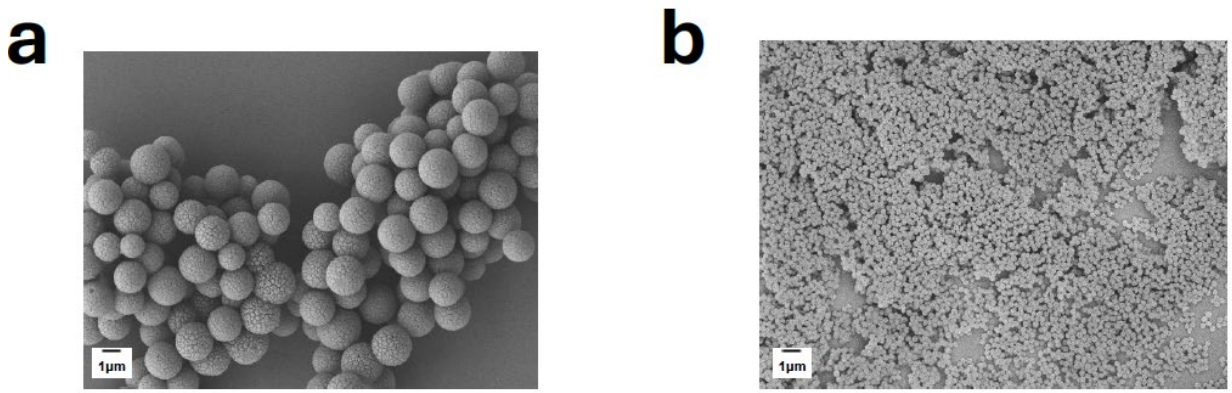

Figure 1. FESEM *images of TAPB-DMTP COF synthesized under solvothermal conditions, highlighting the effect of reaction time on morphology and particle size. (a) After 1 h and (b) 5 h of reaction time.*

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Understanding evolutionary trajectories of SARS-CoV-2 proteins

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The understanding of molecular evolutionary trajectories of emerging viruses such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is fundamental for the design of appropriate therapies. In this context, we investigated evolutionary patterns observed in SARS-CoV-2 proteins, including the development of substitution models that can be useful to make phylogenetic inferences. The reduced protein diversity observed in this virus complicated the identification of evolutionary patterns presenting sufficient statistical support, but the evolution of certain structural properties of these proteins could be predicted.

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Synergistic plasmonic and MOF engineering for enhanced photocatalytic ammonia synthesis

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Photocatalytic ammonia synthesis offers a sustainable alternative to the fossil-fuel-intensive Haber-Bosch process by harnessing solar energy to convert nitrogen (N2) into ammonia (NH_3) under ambient conditions. While this method significantly reduces CO_2 emissions, it faces challenges such as low N2 solubility in water and competition with the hydrogen evolution reaction (HER), which hinder its efficiency and scalability.

Zeolitic imidazolate frameworks (ZIFs), in particular ZIF-8, have been largely used to incorporate nanoparticles with different morphology. However, the photocatalytic performance of these hybrids is limited by the large bandgap of ZIF-8. To overcome these limitations, nickel doping has been used to enhance charge transfer and create additional catalytic sites

In this study, a hybrid system was developed by integrating nickel-doped ZIF-8 with anisotropic Au plasmonic nanoparticles, such as gold nanorods (AuNRs) and gold nanostars (AuNSTs). This combination not only reduces nanoparticle aggregation but also improves reactant accessibility and light absorption under irradiation. Furthermore, nickel inclusion enhances the electronic conductivity and increases the active site density in ZIF-8. Altogether, these hybrid structures led to a substantial enhancement in catalytic performance, with a twofold increase for AuNRs@NiZIF-8 and a sevenfold increase for AuNSTs@NiZIF-8 compared to NiZIF-8. In addition, the catalytic activity remains stable across three consecutive cycles.

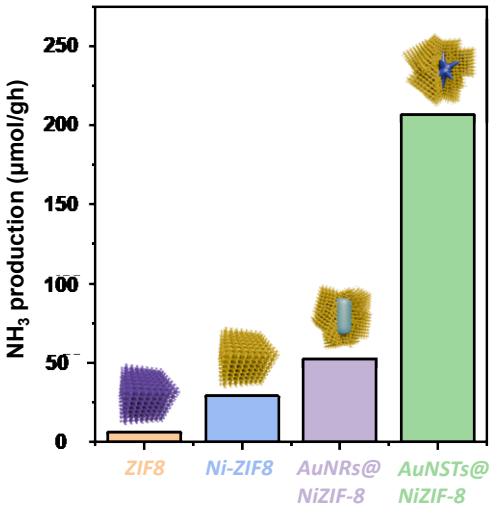

Figure 3. NH_3 yield hybrids.

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Ministerio de Ciencia e Innovación, Ayudas para la formación de profesorado Universitario (FPU).
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Formulation and characterization of ulvan-based films: impact of plasticizer type and concentration on physicochemical properties

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Ulvan, a sulfated heteropolysaccharide extracted from *Ulva* spp. (green macroalgae), has gained increasing interest in the last decade as a sustainable and multifunctional biomaterial [1]. Despite its promising bioactivities (antioxidant, anti-inflammatory, antiviral or anticancer) ulvan remains underexplored and underexploited [2], particularly in the context of film formation. This study focuses on the development and characterization of ulvan-based films using a non-destructive ultrasound-enzyme assisted extraction method, thereby preserving its native chemical structure and biofunctional properties [3].

We formulated ulvan films with various plasticizers, namely glycerol and polyethylene glycol 600 and 2000, at different concentrations and ratios, to assess their influence on film properties. Comprehensive analyses were conducted, including Fourier-transform infrared spectroscopy, elongation at break, rheological behavior (G', G''), swelling degree, opacity, and differential scanning calorimetry. The results highlight significant variations in film flexibility, hydrophilicity, thermal transitions, and structural organization depending on the type and concentration of plasticizer. These findings contribute to the development of ulvan-based biodegradable films with tunable

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Deciphering gaze movements: neural mechanisms for stabilizing and goal-oriented eye movements in lampreys

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To perform advanced behaviors employing vision, mechanisms are necessary to stabilize the scene on the retina. One such mechanism is the optokinetic reflex, which is mediated by the pretectum (PT) (Figure 1) [1, 2]. Additionally, these stabilization responses are modified to redirect gaze towards specific targets, a process in which the optic tectum (OT) plays a crucial role [2]. Here, we investigated the role of PT and OT in processing both optokinetic and non-optokinetic stimuli in the lamprey; an accessible animal model that enables ex-vivo preparations maintaining sensory organs. Furthermore, the stepwise development of its visual system allows to study the role of its underlying neuronal circuits [3]. For this, we employed a combination of anatomical techniques and electrophysiology/eye-tracking in response to visual stimuli to investigate circuit connectivity and functionality (Figure 2). Our results show that larval stages exhibit functional basic light detection movements mediated by the OT and PT via the nucleus of the medial fasciculus, with direct motor outputs to the reticular formation emerging during metamorphosis [4]. In adult lampreys, non-optokinetic visual stimuli elicited responses in both the PT and OT. However, optokinetic stimuli induced sustained activation of neurons in the PT, while activity in the OT was short in duration, potentially due to the activation of the tectal GABAergic system. Additionally, by using pharmacological perturbations, the OT and PT circuits were analyzed to unveil their interaction and individual contribution to gaze responses.

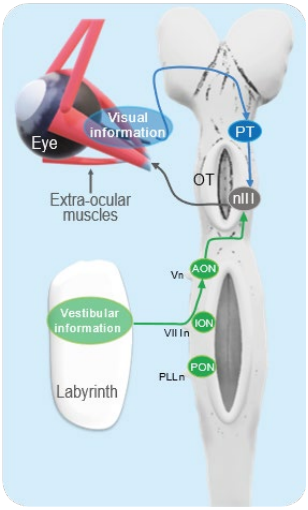

Figure 4. Neuronal circuits under study. Schematic dorsal view of lamprey larvae brain with the circuits that generate VOR and OKR through the dorsal rectus muscle.

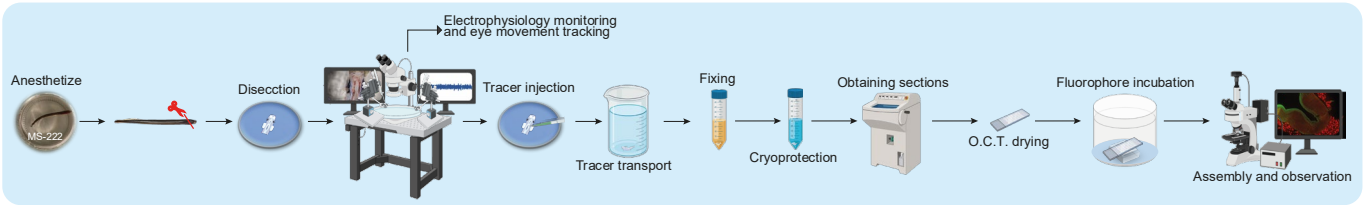

Figure 2. Methods.

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Role of *FAM49B* in the interaction between cytoskeleton and mitochondrial dynamics in models of ciliopathy

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Rare diseases manifesting abnormalities in the structure or function of cilia are grouped under the name of ciliopathies. Bardet-Biedl syndrome (BBS) and Alström syndrome (AS) stand out as two phenotypically similar but genetically different syndromes. Diagnostic features AS is characterised by retinopathy of pigment, obesity, type 2 diabetes, sensorineural deafness, dilated cardiomyopathy, and progressive liver and kidney dysfunction while BBS also has retinopathy of pigment and obesity, but is distinguished by the presence of polydactyly, developmental delay or intellectual disability, renal abnormalities and, frequently, hypogonadism. Recent studies have shown that ciliary dysfunction, as seen in BBS and AS, significantly affects mitochondrial dynamics leading to mitochondrial hyperfusion and fragmentation respectively.

Proteomic analyses suggest a role for the FAM49B protein in the abnormal phenotype displayed by mitochondria in these pathologies. As this protein is presumably located in the mitochondrial membrane and possibly interacts with actin, we wondered whether its role in mitochondrial division and fusion is key in the development of the altered phenotype. This study aims to evaluate the role of the FAM49B protein in knock-out (KO) models in ciliopathies by studying the role of MAMs, specifically in the interaction between the actin cytoskeleton and mitochondrial dynamics by assessing the colocalisation of both organelles in retinal epithelial cells (RPE-1, specifically in *KO* models for *BBS1* and *BBS4* genes, and fibroblasts (BJ5-TA in *BBS1 KO* and *ALMS-1 KO*), by immunofluorescence.

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Recreating a vascularized colorectal tumour-on-a-chip for drug testing

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Colorectal cancer (CRC) ranks as the third most common and second most lethal cancer worldwide, with current treatment regimens increasingly hindered by the development of drug resistance. This highlights the critical need for novel therapeutic strategies. We demonstrated the anticancer properties of a Ruthenium-cyclopentadienyl compound, which significantly inhibited cell proliferation, colony formation, and metabolic activity in SW480 and RKO CRC cell lines using both 2D and 3D culture systems (Fig.1A). However, these traditional in vitro models lack the complexity of the native tumour microenvironment and often fall short in predicting clinical drug responses. To improve clinical relevance, we developed a vascularized CRC-on-a-chip using PDMS and CAD-based microfluidic designs. Computational fluid dynamics simulations of pressure and velocity fields were also performed (Fig. 1B). The chip connects three compartments with vasculature formed by human umbilical vein endothelial cells, normal human fibroblasts, and CRC cell lines. Vascular integrity was assessed using fluorescent microbeads and FITC-dextran via confocal microscopy (Fig. 1C). Drug response will be measured by changes in CRC cell fluorescence area. This biomimetic model holds significant promise for accelerating the development and screening of Ruthenium-based compounds for CRC therapy.

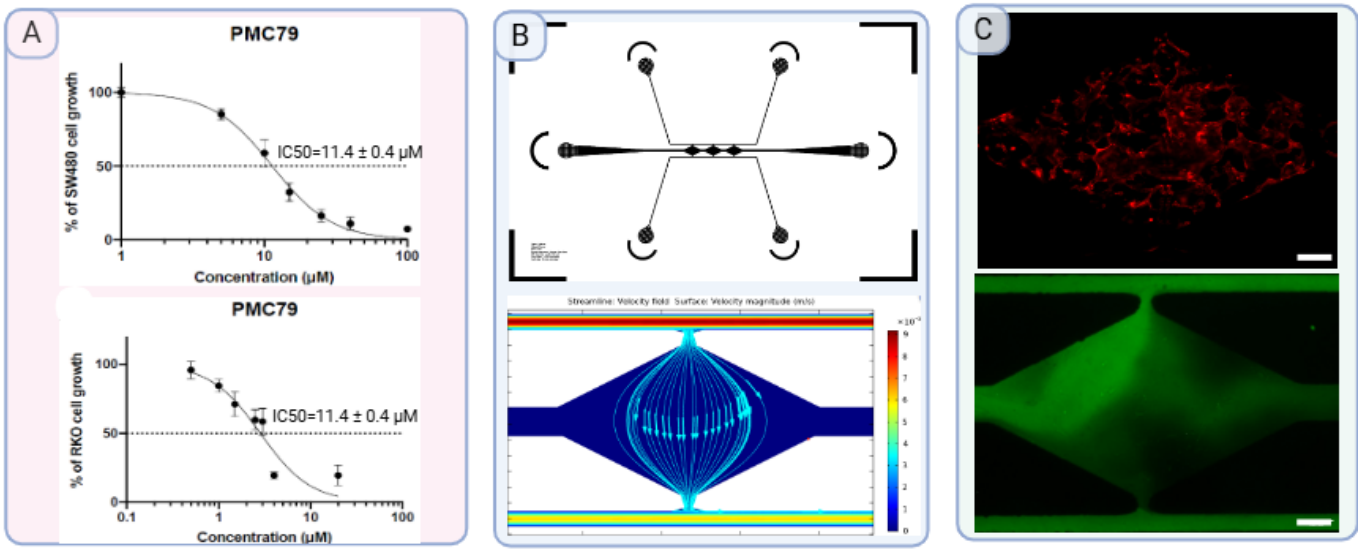

Figure 1. (A) Dose-response curve of Ruthenium-based drug in the micromolar range of toxicity for CRC cell lines. (B) Device schematic and simulated pressure drop field within a tissue chamber indicates that the hydrostatic pressure difference is uniform with an average of 50 Pa. (C) HUVEC-RFP vascular network (top) and 70 kDa dextran diffusion (bottom). Scale bar: 200 µm. (C) Preliminary results of HUVEC-RFP early vascular network in day 2 (top) and 70 kDa dextran diffusion (bottom). Scale bar: 200 µm.

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ENROFLOXACIN ADSORPTION FROM WATER BY A COVALENT ORGANIC FRAMEWORK

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Enrofloxacin is a fluoroquinolone antibiotic widely used in veterinary medicine to treat infections from a broad spectrum of Gram-negative and Gram-positive bacteria, including *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus* [1]. Fluoroquinolones have limited biological metabolization. This, together with the inefficiency of the current wastewater treatments for their removal, are the main causes for their prolonged presence in natural and wastewaters, which has been extensively reported [2]. Therefore, it is essential to find new efficient strategies to remove and monitor the presence of fluoroquinolone antibiotics to maintain water quality and protect public health.

Covalent organic frameworks (COFs) are highly attractive materials for the adsorption of water contaminants due to their crystallinity, large surface area, and pore uniformity [3]. We have previously reported that TpBD-$(CF_3)_2$ COF is an efficient adsorbent for pharmaceuticals in natural waters, including several fluoroquinolones [4]. Herein, we present a systematic study of the performance of this COF in the adsorption of one of most common fluoroquinolones, enrofloxacin.

After the synthesis and characterization of TpBD-$(CF_3)_2$ [5], adsorption experiments were carried out in water at different pH values. The COF performed the best at pH 4 with 78% of enrofloxacin adsorbed. Adsorption kinetics of enrofloxacin onto the COF in ultrapure water showed that equilibrium was reached in 4 h, presenting a maximum experimental adsorption capacity q_e of 28 mg g^{-1}, outperforming the capacity of most of recently reported adsorbents [6]. The isotherms studied showed that the linear form of the Langmuir model explains the adsorption the best (R^2 = 0.925). According this model, the maximum adsorption capacity, q_m = 26 mg g^{-1}, is very close to the experimental one Desorption using isopropanol resulted in a recovery of 80% of the adsorbed enrofloxacin after overnight incubation at room temperature. Recyclability of the COF was demonstrated until 5 cycles of adsorption/desorption, with a decrease of only 25% in the capacity. Overall, these results demonstrate that TpBD-$(CF_3)_2$ is an efficient adsorbent for the extraction of enrofloxacin from water and could be implemented in cartridges for solid-phase extraction for their monitoring.

Acknowledgements: *Fundação para a Ciência e a Tecnologia is acknowledged for funding through project Charm (PTDC/QUI-OUT/2095/ 2021).*

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Structural and Functional Consequences of Drug Resistance Mutations in HIV-1 Integrase

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The high mutation rate of HIV did not allow yet the development of efficient vaccines and favors the emergence of drug resistance mutations against antiretroviral therapies. Understanding the fixation of resistant mutations is relevant to designing efficient treatments. In this regard, despite resistance mutations can allow a fitness advantage in the presence of a drug treatment, they could also affect the stability and binding affinity of the viral proteins with their natural substrates, and understanding this counterbalance is crucial for predicting which mutations are more likely to be fixed. We investigated the influence of common resistant mutations on the folding stability and binding affinity of the HIV-1 integrase (IN) through molecular dynamics simulations. Our results will contribute to a better understanding of the evolution and establishment of virus resistance variants, which may help the development of more effective antiviral strategies.

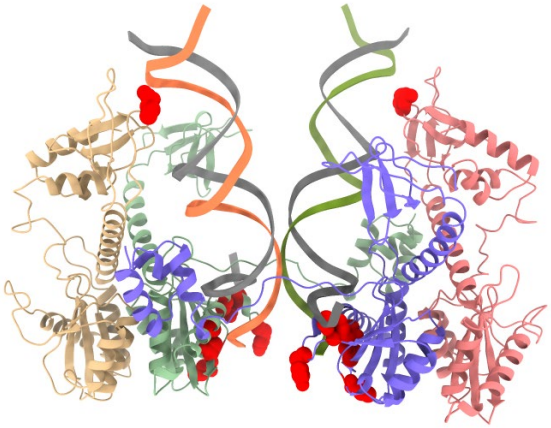

Figure 1. Structure of HIV-1 integrase bound to its natural substrate DNA. Each protein chain is shown in a distinct color for clarity. Clinically significant positions associated with resistance to integrase inhibitors are highlighted in red.

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Photocatalytic Reduction of CO_2 to Ethanol Using AgRu Single Atom Alloys

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The utilization of captured CO_2 as a feedstock for the production of chemicals and fuels is regarded as one key strategy to effectively tackle the global climate change. Ethanol is considered as a particularly attractive target because it serves as a building block for the manufacture of a multitude of chemicals and could serve as an energy carrier in the future. Inspired by the natural photosynthetic process, photocatalytic CO_2 reduction (CO_2RR) emerges as a sustainable alternative to the current fossil fuel-based ethanol production because it operates at ambient temperature and pressure using solely water and renewable solar energy as feedstocks.[1] However, the possible formation of other compounds derived from CO_2, low quantum yields, and slow reaction kinetics remain as crucial challenges for achieving efficient photocatalytic CO2RR.

To increase the solar to energy conversion, different strategies have been investigated over the last decades, including crystal phase and facet engineering, element doping, surface defects creation and heterojunctions, or the addition of co-catalysts and photosensitizers forming heterogeneous hybrids. Recently, plasmonics have attracted much attention for improving the efficiency of photocatalytic CO_2RR, enabling greener and more cost-effective CO_2 photo(electro)reduction.[2] The unique optical properties of metal nanoparticles (NPs) in the visible and near-infrared ranges make them ideal for sunlight-activated catalysis to obtain molecules like formic acid, formaldehyde, carbon monoxide, ethane, methane and methanol. Adding a second metal helps store more electrons to produce complex products, such as hydrocarbons and certain higher alcohols, and optimizing the metal combination can improve selectivity.[3] Single Atom Alloys (SAAs) consisting of one active metal that is atomically dispersed on a more stable but selective metal host, a plasmonic nanoparticle in this case, have gained notable attention owing to their unique structure. They are selective, stable, and resistant to CO poisoning offering a new avenue for the development of efficient materials in catalysis, photocatalysis, and photoelectrocatalysis.

In this context, our work focusses on the development of new SAA silver sphere nanoparticles with isolated ruthenium atoms photocatalysts for the CO_2 reduction to ethanol. We obtain a catalyst able to selectively produce ethanol under solar irradiation using water as the sole solvent, that in addition, is recyclable.

The authors acknowledge the financial support from the Spanish Ministry of Science and Innovation under the TED2021-132101B I00 project.

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A new green approach to photocatalytic nanomaterials

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The introduction of mesoporosity in amorphous silica using surfactants was revolutionary in the silica materials industry. This breakthrough expanded its range of applications - due to enhanced surface area - making it a suitable base material for drug delivery, opto-electronics, catalysis and photocatalysis, and beyond [1], [2]. However, industrial production of synthetic mesoporous silica commonly relies on resource-intensive international supply chains [3]. Therefore, novel approaches toward more sustainable and locally produced alternatives are needed.

Diatoms, the most abundant single-celled microalgae group on Earth, could function as an alternative. These microorganisms possess highly porous intricate silicon dioxide cell walls, called frustules. Diatoms are the most diverse group of algae, with over 100 000 estimated species [4]. Their frustule morphology is species-specific, varying in overall shape (pennate and centric), size range (2 - 500 µm) and nanopore patterning. The potential utilization of diatoms as a naturally available nanomaterial has already been suggested for different types of advanced application [5], [6].

One of the most promising methods currently available for environmental remediation is photocatalysis, valued for its low activation energy requirements, low-cost chemicals (although often environmentally costly produced), and recyclability [7]. Mesoporous silica, facilitating a wide range of photocatalytic applications, is a state-of-the-art field of research. Diatom biosilica has been suggested as a photocatalytic support material [8], [9], however the exploitation of species-specific characteristics for targeted applications was only barely studied.

In this project, I will develop a new class of innovative silicon dioxide nanodevices by utilizing the unique species-specific characteristics of diatoms. The project will target primarily photocatalytic applications, reducing reliance on synthetically and environmentally unfriendly produced materials. I will use of the natural ability of diatoms to integrate materials from the environment, to functionalize the frustule with relevant catalytic agents. These nanodevices will be fabricated and evaluated through proof-of-concept experiments to demonstrate their potential – primarily as novel template materials for photocatalytic platforms, with the capacity to advance emerging technologies beyond the current state of the art for societal benefit.

This research is supported by the FCT – Foundation for Science and Technology, under PhD studentship number 2024.01564.BDANA.

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SERS in action: Metabolomic Profiling of Breast Cancer Cells

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Breast cancer is the most common type of cancer worldwide and the second leading cause of cancer-related deaths among women [1]. Metastasis accounts for over 90% of these deaths and occur when circulating tumor cells (CTCs) escape from the primary tumor to distant organs through the bloodstream or lymphatic system. Given heterogeneity in metastatic potential among CTCs, studying individual cells is crucial for uncovering the mechanisms driving metastasis [2].

Recent studies have demonstrated that combining three-dimensional (3D) cell culture and droplet-based microfluidic systems with surface-enhanced Raman scattering (SERS) spectroscopy, which is a highly sensitive variation of Raman spectroscopy, enables the label-free analysis of extracellular metabolites in culture media [3][4]. However, current strategies for real-time, single-cell metabolomic profiling are still limited, and there is a need to develop new approaches.

Thus, this work proposes a new approach to develop a real-time sensing strategy for extracellular metabolomic analysis during the proliferation of single CTCs into spheroids. For this purpose, gold nanostars (GNSs) were synthesized, characterized, and labelled with 1-Naphthalenethiol, a well-known Raman reporter, and different metabolites, to demonstrate their potential as SERS sensors and were also encapsulated in microdroplets to show their applicability in droplet-based sensing systems. These nanosensors were integrated in 3D dome culture model to enable label free, continuous *in situ* monitoring of metabolites secreted by different cell lines over time. By analysing the SERS data using machine learning, this approach will allow the identification of distinct metabolomic profiles associated with normal versus breast cancer cells at different time points, offering critical insights into metastatic processes and potentially contributing to the advancement of personalized medicine.

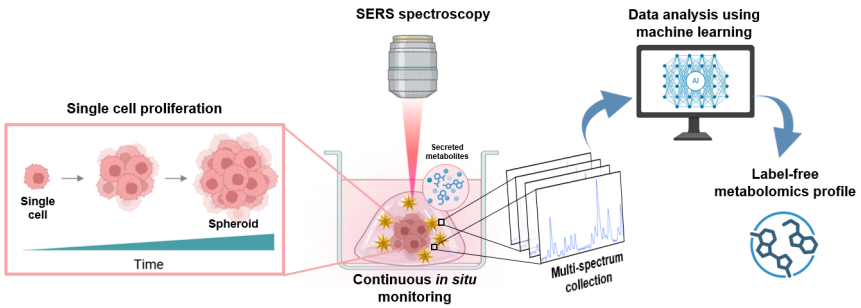

Figure 1 – Scheme illustrating the workflow of the continuous SERS analysis for the identification of distinct metabolomic profiles associated with different cell lines. Scheme created on BioRender.

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Exploiting Connexin 43 to Enhance NK Cell-Mediated Antitumor Immunity

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NK cells are key effectors of the innate immune system with potent anti-tumor activity, making them promising candidates for cancer immunotherapy due to their ability to rapidly kill tumor cells without prior sensitization or antigen recognition. CAR-NK cells are engineered to express chimeric antigen receptors (CARs) through genetic modification, enabling them to recognize specific surface antigens on target cells. While CAR-NK therapy builds on the principles of CAR-T therapy, it also leverages the unique biological properties of NK cells. Connexins (Cxs) are transmembrane proteins that form gap junctions (GJs), allowing direct intercellular communication between tumor cells and other components of the tumor microenvironment (TME). Gap junction-mediated interactions between tumor and immune cells have been shown to improve immune recognition and tumor clearance. In fact, Connexin43 (Cx43) has been reported to be part of the immunological synapse between tumor and immune cells. In our group, we have identified Cx43 as an enhancer of NK cell cytotoxicity, including in a prototype CAR-NK model, improving their ability to recognize and kill tumor cells. We now aim to investigate the mechanism of action through which Cx43 enhances NK cell killing activity, as well as the potential involvement of other connexins in this process. In this study, we first analyzed the gene expression profile of connexins in a human NK cell line. Cx43 emerged as the most highly expressed connexin, along with Cx62 and the Cx43 pseudogene. Cx43 was predominantly detected as post-translationally modified isoforms (potentially SUMOylated). Subcellular fractionation and Western blot analysis revealed the presence of native Cx43 (43 kDa) in membrane extracts, indicating localization consistent with gap junction and hemichannel formation. Functional assays demonstrated that NK cells can form gap junctions with tumour cells, regardless of Cx43 expression, although intercellular communication was significantly more efficient with tumour cells overexpressing Cx43. These findings suggest that although NK cells express low levels of Cx43, they retain the capacity to form functional GJs with tumour cells, and enhancing Cx43 in tumour cells increases NK cell-mediated cytotoxicity. Our results support the potential use of Cx43 to enhance NK-tumor cell communication and potentiate NK cell-mediated tumor eradication.

Designing Plasmonic Nanoparticle@UiO-67-bpy-Cu Catalysts for Efficient CO_2 Photoreduction to Methanol

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Photocatalytic conversion of CO_2 into useful chemicals and fuels using sunlight offers a sustainable route to mitigate greenhouse gas emissions while producing value-added products such as methanol, a key chemical intermediate and energy carrier. Although promising, photocatalytic methanol production still suffers from limited efficiency and selectivity. To overcome these limitations, we developed a hybrid photocatalyst based on the integration of copper active centers and plasmonic gold nanospheres (AuNSp) within a metal-organic framework (MOF).

Au@UiO-67-bpy MOFs synthesized using a core-shell strategy were post-modified with copper ions through coordination with bipyridine sites, creating a uniform distribution of active catalytic centers.

Under visible light irradiation, the Cu-functionalized MOF (UiO-67-bpy-Cu) catalyzed the selective reduction of CO_2 to methanol without requiring a sacrificial hole scavenger. Remarkably, embedding gold nanospheres into the framework led to a twofold increase in methanol yield compared to the Cu-only system. This enhancement is attributed to a synergistic effect between hot electron injections, arising from intra- and inter-band transitions in the plasmonic gold, and the redox activity of the copper centers. (Fig.1)

The catalyst showed excellent stability across four consecutive reaction cycles, with consistent performance and methanol selectivity above 90%, and no detectable structural degradation. [1] These findings highlight the promise of plasmon-enhanced MOF systems as robust and efficient platforms for solar-driven CO_2 conversion to methanol.

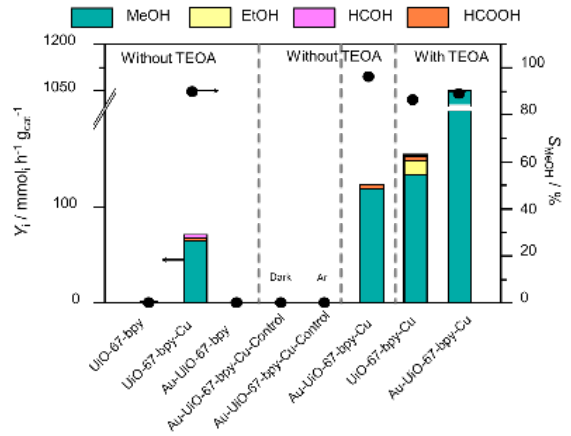

Figure 1. Catalytic performance toward methanol production under reaction conditions, with relevant controls.

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Antimicrobial peptide-functionalized nanoparticles: production and characterization

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Treatment of infected wounds with antimicrobial peptides (AMPs) is not yet a reality, due to their instability *in vivo*. Among other strategies, AMP grafting onto nanoparticles (NPs) has been proposed to overcome peptide aggregation, precipitation and proteolytic degradation, while enabling a better AMP exposure and a faster antimicrobial action [1]. In this work, a thiolate version of MSI78 (commercially known as Pexiganan®), a broad spectrum AMP [2], [3], was immobilized onto poly(D,L-lactide-co-glycolide)-polyethylene glycol (PLGA-PEG) NPs. NPs were prepared by nanoprecipitation using a ratio of PLGA-PEG/PLGA-PEG-Mal (40% PLGA-PEG-Mal) and MSI78-SH (1.5 mg/mL) was covalently grafted through thiol-maleimide reaction, as described in [1]. NP were characterized regarding their size, polydispersity index (PDI) and zeta potential using Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS), respectively. Grafted AMP was quantified using the fluorescamine assay. [1] Free MSI78-SH and MSI78-grafted NPs were tested against *Pseudomonas aeruginosa* (ATCC27853), one of the most prevalent bacteria in infected wounds, for the determination of Minimum inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC). Bare NP average size was 112±1nm and MSI78-grafted NPs average size was 208±1nm. Both NPs exhibited a monodispersed population with a PDI of 0.1 for bare NPs and 0.25 for MSI78-grafted NPs. The zeta potential increased from -7±0.2mV (bare NP) to -0.2±0.1mV (MSI78-NP), confirming the presence of the cationic AMP. The amount of MSI78 grafted onto NP surface was around 386 µg/mL, corresponding to 25% of immobilization efficiency, which is consistent with previously reported values for other AMPs [1]. MSI78-SH showed a MIC of 4-8 µg/mL and a MBC of 8 µg/mL against *P. aeruginosa*. Antimicrobial assays for the MSI78-NP are currently being performed.

The modification of the AMP with the thiol (MSI78-SH) did not affect, significantly, the antimicrobial activity. MSI78-SH was successfully grafted onto PLGA-PEG NP with high immobilization efficiency and presenting size, PDI and charge values consistent with previous reports supporting their potential for antibacterial activity.

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Isolation and Characterization of Phages from Environmental Settings and Their Therapeutic Potential Enhanced by Nanomedicine

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Cystic fibrosis is a hereditary disorder that predisposes patients to chronic pulmonary infections due to the favorable environment the lungs provide for microbial colonization. Commonly isolated pathogens include *Pseudomonas aeruginosa*, the *Burkholderia cepacia* complex, and *Mycobacterium abscessus*, among others[1]. *Mycobacterium abscessus* displays intrinsic resistance to most conventional antibiotics, often requiring prolonged treatment with agents such as isoniazid and rifampicin, which carry a significant risk of hepatotoxicity.

Bacteriophages (phages), viruses that specifically infect bacteria, are abundant in diverse ecosystems and offer promising therapeutic potential owing to their host specificity, capacity for self-replication, low toxicity, and effectiveness against bacterial biofilms. Despite these advantages, clinical application remains restricted to compassionate use, largely due to challenges in dosing, immune system interactions, and limited public and clinical familiarity[2].

Nanotechnological strategies, including phage encapsulation within liposomes or nanoparticles, present a viable means to overcome these limitations. These formulations can enhance phage stability in physiological conditions, improve targeted tissue delivery, prolong systemic circulation, and reduce immune clearance[3] (*Figure 1*).

In this study, we isolated phages targeting mycobacteria from sewage and established a phage library. Several phages demonstrated lytic activity against *M. abscessus* and were screened against clinical isolates. The most effective candidates were characterized using electron microscopy and whole-genome sequencing. Growth kinetics were assessed individually and in combination to formulate optimized phage cocktails for future therapeutic encapsulation, aiming to improve delivery, achieve sustained release, and enhance overall treatment efficacy.

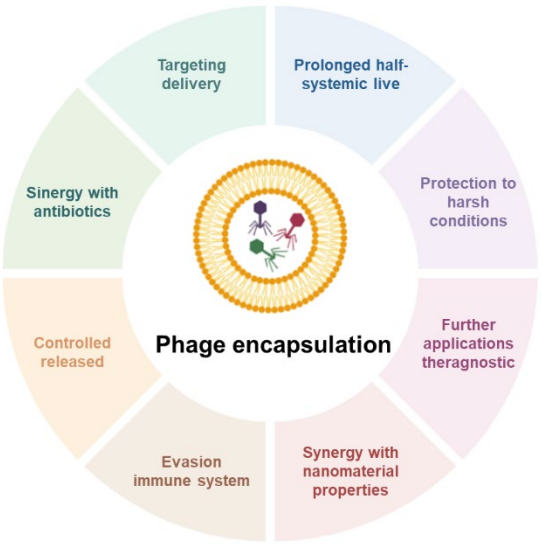

Figure 5. Main advantages of encapsulation of phages.

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Single cell-derived spheroids as a model to characterize CTC heterogeneity in breast cancer

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Breast cancer (BC), the second most commonly diagnosed cancer globally with 2.3 million new cases in 2022, is a highly heterogeneous disease.[1] Metastasis - a complex process involving cell detachment from the primary tumour, invasion, intravasation into the bloodstream, and colonization – leads to the spread of cancer.[2] Cells that enter the bloodstream from solid tumours are known as circulating tumour cells (CTCs). Although metastasis significantly impacts patients' long-term survival, it remains inefficient, with only a small fraction of CTCs capable of surviving in circulation and successfully establishing secondary tumours.[3]

In this study, MCF-7 cells – a model for luminal A subtype of BC, which predominates in our patient cohort - were used as a surrogate for primary tumour cells. These cells were cultured within an 8-µL dome of human adipose tissue-derived extracellular matrix (adECM) and cultured for 28 days (Figure 1).[4] Optimizations of culture conditions, such as culture media, hypoxia, were performed to achieve the optimal culture conditions. For studies with primary cells, 7.5-mL whole blood from metastatic BC patients was processed through a RUBYchip®, a microfluidic device that isolates CTCs based on their size and deformability. Captured cells were subsequently recovered and cultured in the same conditions optimised for MCF-7 cells. Our results demonstrate the ability of individual BC cells to form 3D multicellular spheroids, providing a platform to study metastatic potential. Ongoing research aims to isolate CTCs from the blood of a cohort of 80 BC patients to explore correlations between CTC phenotypes and clinical outcomes, with the goal of identifying new drivers of BC metastasis.

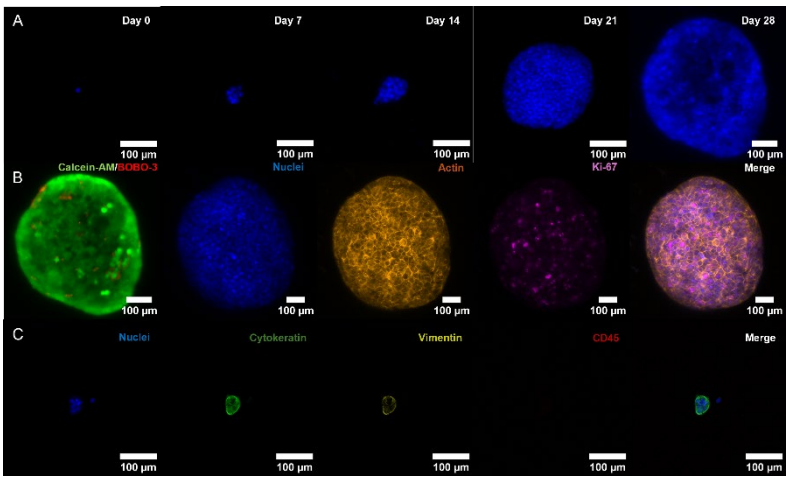

Figure 1. Breast cancer spheroids, each derived from a single MCF-7 cell. A) Time-course imaging of spheroid growth, showing nuclear staining from a single cell at day 0 to hundreds of cells by day 28. B) Live/dead assay (left) and immunofluorescence staining for nuclei, filamentous actin, and Ki-67 (right) in two spheroids at day 28. C) MCF-7 cells captured using the RUBYchip® were successfully recovered and cultured, resulting in spheroid formation (14 days).[4]

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AIChemiSSts: Alternative Chemicals and Materials integrating Safety, Sustainability, new Production technologies and Socio-economic aspects

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Substances of very high concern (SVHC) [1], according to the Article 59 (10) of REACH Regulation [2], have serious effects on human health and the environment, which is the case of medium chain chlorinated paraffins (MCCPs) and antimony compounds used in flame retardants (FRs), zinc salts and naphthenic acids used as surfactants (SFs), and low molecular weight ortho-phthalates used as plasticizers (PCs) [3]. To promote the substitution of SVHC, the European Commission (EC) has introduced the Safe and Sustainable by Design (SSbD) framework [4]. The SSbD framework is based on a holistic approach proposed by the EC's Joint Research Centre (EC-JRC) [5] to comprehensively assess the safety and sustainability of chemicals and materials throughout their life cycle, including SSbD criteria and implementation mechanisms.

The main goal of the AIChemiSSts project is to test and demonstrate the applicability of the SSbD framework to develop innovative chemicals or materials to replace SVHCs in high-impact markets, notably for surfactants, plasticizers, and flame retardants in relevant value chains, including metal working fluids (MWFs), lubricants, insulation foams and paints, safety boots and wellies, battery cases, and sports mats. Through a structured approach that spans from laboratory innovation to industrial demonstration, the project promotes sustainability, safety, and circularity while minimising environmental and health impacts.

Our group performs and coordinates the experimental environmental hazard assessment of the materials and products developed by the project value chains. The INL team also helps to establish the operational framework, providing new data for the data hub and ensuring the application of FAIR principles.

Acknowledgement:

The authors acknowledge the financial support of the project AIChemiSSts, which receives funding from the European Union's Horizon Europe research and innovation programme under grant agreement 101178074.

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Development of a microfluidic platform for the autonomous production of chiral gold nanorods

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The use of nanoparticles in biomedical applications has been gaining momentum in the last few years, allowing not just for their use as enhanced systems for drug delivery and localized cancer treatment1, but also in the improvement of existent characterization techniques for chemical composition. One of those techniques, Raman spectroscopy, has greatly beneficiated from the use of metallic nanoparticles (such as gold or silver) to augment the strength of its signal caused by inelastic light by several orders of magnitude, also known as surface-enhanced Raman spectroscopy (SERS)2. The production of nanoparticles using microfluidics has been presented as a novel method of production of nanoparticles in sealed reservoirs, by taking advantage of the multiple advantages that microfluidics permits when it comes to controlled chemical reactions3.

In most nanoparticle synthesis techniques, the process is performed by subsequent steps being taken in specific time intervals and with specific conditions, being difficult to recreate the same settings in microfluidic chips. For this work, we intended to replicate an existent and confirmed method for the synthesis gold nanorods with chiral growth dynamics4 in a microfluidic setting, by adjusting the required conditions for its controlled growth and applying them in a simplified microfluidic chip design by controlling the concentration of reagents, the flow rate of the reagents introduced and the time given for the mixing to occur within the microfluidic channels. For the mixing of the reagents and formation of droplets, two different microfluidic moulds were used. In order to provide enough time for the reagents to mix inside the microfluidic channels by diffusion, the flow rate for the syringe pump of the 5 reagents was adjusted to 168 µL/hr and the flow rate for the HFE 7500 oil (continuous phase) was set at 210 µL/hr. The CTAC and Gold salts ($HAuCl_4$) were injected into a flow focusing mould with 2 inlets and an outlet with 100 x 80 µm cross-section then left mixing for 15 min inside an extension of PTFE tubing placed on top of a hot plate at 40ºC, and then injected into the main microfluidic mould with 4 inlets and 1 outlet, mixed along with gold nanorods, L-cysteine (L-cys) and Ascorbic acid (AA). The 5 reagents were left mixing in a similar way to the initial mixing of CTAC and Au salts inside an extension of PFTE tubing for 30 min at 40ºC, The preliminary results demonstrate that gold nanorods obtained an aspect ratio closer to the aspect ratio associated to the enlarged gold nanorod after the 1-step chiral growth process that to the normal gold nanorod sample.

Acknowledgements: this work has been funded by Health from Portugal (C630926586-00465198), through the NextGenerationEU Fund.

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Chiral Metasurfaces Unlock Strong and Tunable Circularly Polarized Emission in Perovskite Nanocrystals

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Chiral nano-emitters have gained significant research interest due to their potential in advanced technologies and the need to understand the fundamental relationship between structure and properties at the nanoscale. Among them, lead halide perovskite nanocrystals (LHP NCs) stand out for their remarkable optical properties and promise in producing chiral emission. However, achieving high-anisotropy circularly polarized photoluminescence (CPL) from intrinsically achiral perovskite NCs remains a major challenge. While chiral ligands have been employed to induce chirality, the resulting anisotropy factors (glum) typically remain low (10^{-3}–10^{-2}).

In this work, we demonstrate a scalable strategy to generate strong CPL from LHP NCs by integrating them with chiral metasurfaces, specifically, 2D gammadion-shaped photonic structures.[1] This approach achieves a record-high glum of 0.56, marking a significant leap for perovskite-based CPL. Additionally, we compare the effectiveness of high-index dielectric versus metallic chiral metasurfaces in enhancing chiral emission. Notably, by combining both types of metasurfaces, we also demonstrate the simultaneous generation of multi-wavelength circularly polarized light, opening avenues for multiplexed chiral photonic applications.

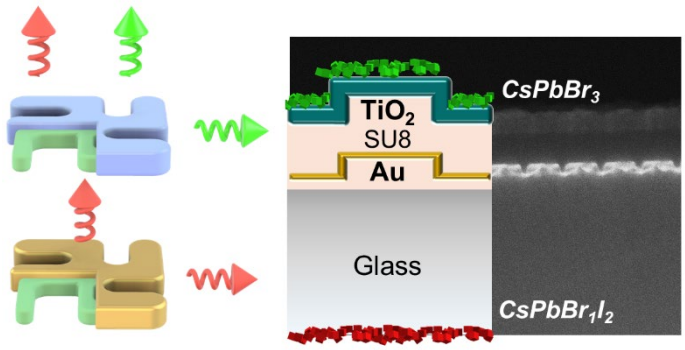

Figure 1. Chiral metasurface architecture combined with perovskite nanocrystals.

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Use of nanoemulsions in photodynamic therapy for ovarian cancer treatment

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Ovarian cancer is a leading cause of cancer death among women, responsible for approximately 207,000 deaths annually worldwide. This high mortality rate is largely due to late-stage diagnosis because of unspecific symptoms that overlap with other conditions [1]. Standard treatments for advanced disease usually include surgery followed by adjuvant chemotherapy, which often results in severe side effects [2]. Photodynamic Therapy (PDT) using photosensitizer-loaded nanoemulsions (NEs) has emerged as an alternative approach offering selective targeting cancer cells while minimizing systemic toxicity.

In this context, we propose the formulation of oil-in-water NEs by spontaneous emulsification using Verteporfin (VP) as the photosensitizer, a molecule that is activated under near-infrared (NIR) light exposure and causes cell death. Two different NEs were developed to load VP based on distinct fatty acid core compositions: oleic acid and miglyol. The hydrophobic properties of VP enabled its encapsulation at various concentrations, and Encapsulation Efficiency was quantified by using spectrophotometric analysis. Physicochemical characterization was done by different technologies such as Transmission Electron Microscopy, Nanoparticle Tracking Analysis, and Dynamic Light Scattering to determine the concentration of VP-NEs, their size, their polydispersity index, and their charge. Further, these parameters were used to control its stability at different environmental conditions related to temperature and light exposure. In vitro assays on SKOV-3 ovarian cancer cells were performed not only to demonstrate its internalization but also to evaluate the cytotoxic effects related to NIR light activation.

The physico-chemical characterization enabled optimization of the formulation process while in vitro assays demonstrated efficient cellular uptake and confirmed the potential of these systems for their use as photodynamic therapy in ovarian cancer. Overall, the different NEs showed differences in terms of cellular viability, leading to an improvement of PDT when using miglyol-based NEs due to their higher capacity of drug loading and their lower intrinsic cytotoxic effect.

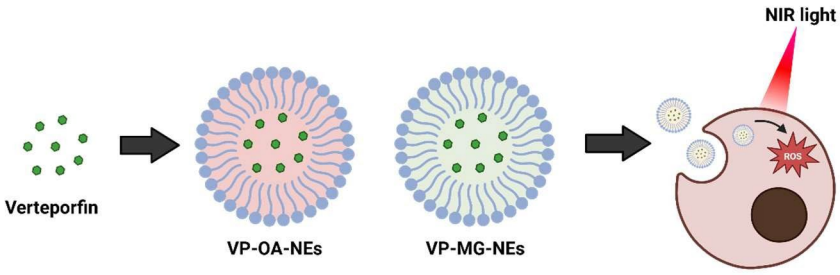

Figure 1. Encapsulation of photosensitizer Verteporfin into oleic acid-based (VP-OA-NEs) and miglyol-based (VPMG-NEs) nanoemulsions to enhance cellular uptake and, therefore, photodynamic therapy effect.

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Cholinergic modulation of TREK potassium channels in the mouse intracardiac ganglia

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The intracardiac nervous system is a parasympathetic neuronal structure composed of diffuse neuronal ganglia, the intracardiac ganglia (ICG), which plays a crucial role in the regulation of cardiac physiological functions [1].

TREK channels, belonging to the two-pore domain potassium (K2P) channels family, maintain the resting membrane potential and control the excitability [2]. These channels are characteristically modulated by a variety of mechanical and chemical stimuli, including the second messenger pathway of muscarinic G-protein coupled receptors [3]. As this modulation influences the membrane potential and neuronal excitability and can affect the cardiac regulation, we wanted to assess the relevance of TREK channels in this process.

In this work, we electrophysiologically characterized the ICG neurons and studied whether TREK channels are modulated by this second messenger pathway using the patch-clamp technique (perforated patch-variant). In current-clamp configuration, the resting membrane potential of ICG neurons was studied, as well as their firing rate. Furthermore, we pharmacologically isolated TREK currents and studied their activation among muscarinic receptor activation. Our results indicate that muscarinic receptor agonists do not regulate, at least directly, the activity of TREK currents. This suggests that they might be activated through other second messenger pathways to control the neuronal excitability of ICG neurons.

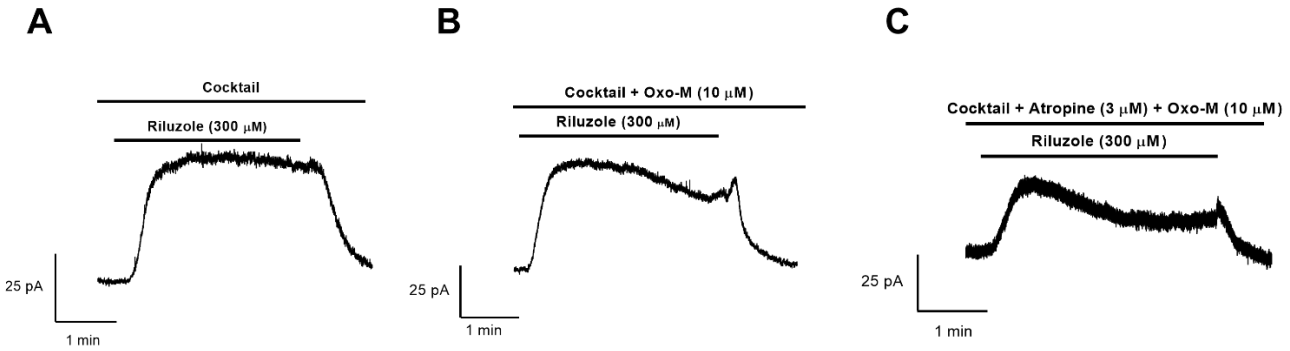

Figure 1. Riluzole-activated TREK currents in the presence of (A) riluzole, (B) agonist oxo-M and (C) muscarinic antagonist atropine.

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Multiscale Simulations of Water Splitting

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In our research in the nanoworld, we often have to deal with reactions and other processes, such as plasmon excitation or diffusion of reactants, that happen in a very wide range of lengths and times. A multiscale simulation aims to combine different levels of theory [1] (from ab initio calculations to classical continuum methods) in order to describe the properties and/or the behaviour of a given system, by applying each level of theory to a certain scale of length and time and using the output of one level as the input of the next [2]. In the field of photocatalysis, multiscale simulations can be very useful to further understand the mechanism and time evolution of light-mediated reactions. In particular, the application of a multiscale approach to the photocatalytic water splitting reaction, which plays a crucial role in the mechanism of interesting chemical processes, such as reactive oxygen species (ROS) generation and nitrogen reduction reaction (NRR), can help to discern, with greater detail, its thermodynamic, kinetic and light-dependent properties.

The multiscale approach we aim to develop makes use of ab initio density functional theory (DFT) methods for the computation of thermodynamic properties for each reaction step in the water splitting mechanism; and semi-classical methods, such as the finite element method (FEM), for the analysis of the optical and thermal properties of the studied photocatalyst and the time evolution of the reactions. In order to connect both the quantum and semi-classical models, we use a kinetic approach based on the Eyring equation for the computation of the different kinetic constants of each reaction step.

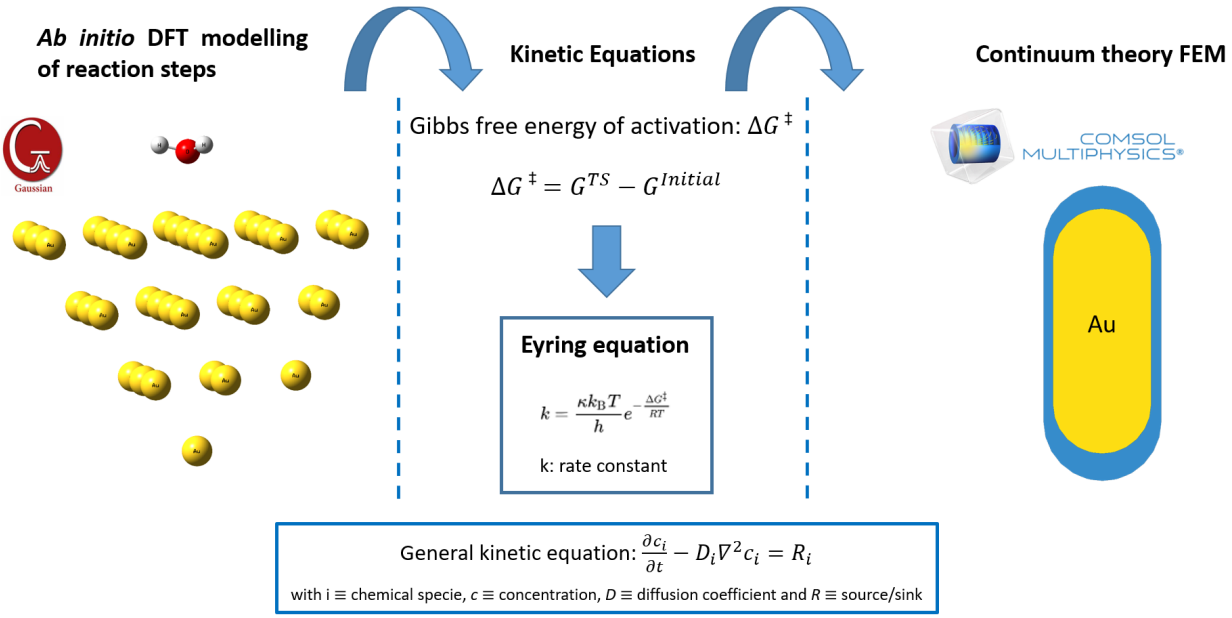

Figure 1. Depiction of the three steps followed by our multiscale simulation approach.

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Products derived from medicinal mineral water for cosmetic uses

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Medicinal mineral water is a natural resource present on our planet and has been used for various purposes since ancient times. Medicinal mineral water springs up on all continents, and due to hydrogeological processes, it is charged with various elements during its time within the Earth. Seawater [1]. It is also a natural medicinal mineral water whose composition encompasses most of the chemical elements.

Medicinal mineral waters possess numerous pharmacological properties [2], due to their chemical composition and physical properties [3]. Many of the properties of medicinal mineral water are beneficial for dermatological treatments [4], primarily due to its moisturizing and soothing effects. Numerous articles in the literature demonstrate the cosmetic effects of mineral-medicinal water [5,6]. This led to a considerable increase in the use of mineral-medicinal water in thermal spas for cosmetic purposes.

The characteristics of mineral-medicinal water mean that it is now an important ingredient in numerous cosmetic products [3], including creams, shampoos, soaps, etc. One of these products derived from mineral-medicinal water is thermal peloids [7], which have been used for cosmetic purposes in various thermal spas around the world, as demonstrated at the recent Ibero-American Peloid Congress held at the Arnedillo Spa in May 2025 [8].

Mineral water products contain elements of high cosmetic value, such as selenium, calcium, magnesium, zinc, and other trace elements [9], which give them high added value.

This paper presents the mineral water products currently in use around the world.

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Post-synthetic Stabilization Strategies for Amine-linked COFs in the Adsorption of Pharmaceutical Pollutants from Water

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The persistence of pharmaceutical residues in aquatic environments poses a critical threat to both ecosystem integrity and human health. Conventional water treatment processes often fail to remove these micropollutants, which can accumulate at µg/L levels in rivers and estuaries. Covalent Organic Frameworks (COFs) have attracted significant attention as adsorbent materials for pharmaceutical micropollutants in water, thanks to their high porosity, crystallinity and chemical modularity. In particular, amine-linked COFs can be prepared under mild conditions and tailored via post-synthetic modification. However, the imine linkage is susceptible to hydrolysis resulting in framework degradation, loss of order and leaching. To overcome these limitations and improve uptake of more hydrophobic drug molecules while preserving framework integrity and recyclability, three post-synthetic stabilization routes have been explored. First, imine bonds are oxidatively converted to amide scaffolds via NaClO2-mediated cleavage1, imparting hydrolytic resistance. Second, an acid-catalyzed Povarov reaction2 enhancing rigidity and hydrophobicity. Third, stepwise thermal cyclocondensation with elemental sulfur3 yields thiazole units, boosting chemical robustness and selective affinity for hydrophobics pharmaceuticals.

For evaluation, adsorption cartridges and SPE columns based on the modified COFs will be tested using estuarine water samples collected from the Tagus River, and their performance will be compared against conventional SPE. The resulting materials could offer promising avenues for the development of durable, reusable extraction cartridges and SPE columns for in situ monitoring and removal of emerging pharmaceutical contaminants.

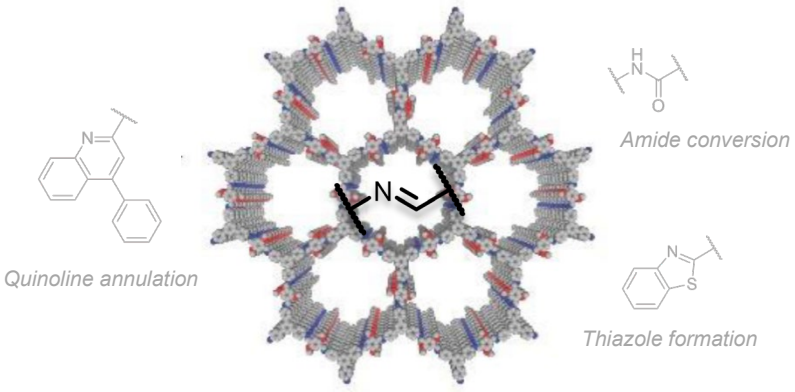

Figure 1. Schematic representation of post-synthetic modification strategies applied to an amine-linked COF.

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A Covalent Organic Polymer-based Electrochemical Sensor for Saxitoxin detection

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Saxitoxin (STX) is a potent and fast-acting neurotoxin produced by cyanobacteria in freshwaters and by dinoflagellates in marine waters. It is one of the major causing agents of paralytic shellfish poisoning, the symptoms of which range from numbness, headache, muscle weakness, and respiratory failure ultimately to death [1]. Currently available methods for STX detection are based on mouse bioassay, high-performance liquid chromatography–mass spectrometry and enzyme-linked immunosorbent assay. However, these present significant limitations, including ethical concerns, high cost, requirement of complex equipment, and/or limited reagents stability [1]. The World Health Organization (WHO) has established a guideline value of 3 µg/L (10 nM) for STX in drinking water [2], highlighting the urgent need for sensitive, reliable and field-deployable detection methods. This work presents a novel portable electrochemical sensor for STX detection based on covalent organic polymers (COPs). Specifically, a carboxyl-functionalized COP (TpPa-COOH COP) was used due to its high adsorption rates for STX [3], excellent chemical stability, and reusability, characteristics that make it well-suited for portable sensor applications. Gold nanoparticles were grown on the COF to gain access to a composite. The resulting composite was used to modify the surface of electrochemical sensors, which were further characterized by scanning electron microscopy, confocal Raman microscopy, electrochemical impedance spectroscopy (EIS) and voltammetry. STX detection was performed by EIS and the developed sensor demonstrated a linear response in the range 3–10 µM, with a limit of detection (LOD) measured at 2.42 µM. These sensors are well-suited for integration into a portable device, offering a promising solution for real-time STX monitoring in environmental water samples. Nonetheless, further development are required to lower the LOD by increasing COP coverage on the sensor surface, in order to enable efficient and continuous assessment of water quality.

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Synthesis and Pharmacological Characterization of a Novel Cannabinoid Receptor 1 Antagonist

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The endocannabinoid (eCB) system regulates several brain functions and is implicated in numerous conditions affecting the brain. Thus, the pharmacological blockade of cannabinoid receptors has therapeutic potential but produces severe psychiatric side effects. Hence, new cannabinoid compounds with different pharmacological profiles are needed to potentially minimize this toxicity.

A series of novel antagonists/inverse agonists of cannabinoid receptors have been developed. The most promising one, UVI3502, has shown affinity for two [^{3}H]CP55,940 binding sites (IC_{50Hi} 0.026 ± 0.43 nM and IC_{50Lo} 772 ± 49.40 nM, R^2 = 0.59) in the rat cortex. Binding assays performed in cannabinoid receptor 1 (CB1) and CB2 overexpressing membranes confirmed moderate affinity for both receptor subtypes, about 10-fold higher for the first one.

Functional [^{35}S]GTPγS assays demonstrated that UVI3502 behaved as an antagonist of CB1 receptors, blocking the stimulation evoked by the potent cannabinoid receptor agonist CP55,940. The *in silico* characterization of the binding to the CB1 receptor through molecular docking and molecular dynamics suggests that this activity could be explained by the planar and rigid structure of UVI3502.

In summary, UVI3502 has proven to be a promising novel antagonist of CB1, making it a new pharmacological tool for the study of the eCB system. These promising results will lead to synthesize a new series of novel antagonists/inverse agonist for blocking cannabinoid receptors in the central nervous system.

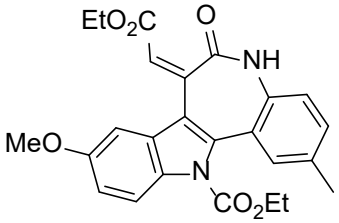

***Figure 1**. Chemical structure of UVI3502*

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Mining Hundreds of Thousands of Sequence Reads from Whole Genome Sequencing of SARS-CoV-2 and HIV-1 to Uncover Recombination Events using the Four-Gamete Test

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Viral intra-host recombination has remained virtually invisible to genomic surveillance because standard analyses rely on consensus genomes –unrealistic pseudosequences that show only predominant variants. Mining around 150 000 SARS-CoV-2 short read sequencing datasets that cover every wave of the pandemic, with a recombination detection framework that we developed based on the four-gamete test directly applied to raw next-generation sequencing reads, we investigated intra-host viral recombination, thus considering the signal of rare viral variants. The results provided a rich catalogue of hidden recombination events and traced high-resolution recombination landscapes across the entire viral genomes (Figure 1). The adaptability of our framework was showcased in a parallel scan of around 2 000 HIV-1 short read sequence datasets, where it retrieved numerous allele combinations concordant with recombination. Indeed, control simulations showed high sensitivity and low error rate in our framework, albeit contingent on the genetic diversity present in each sample. These findings showed that RNA viruses generate chimeric genomes more frequently than those previously detected from analyses of consensus genome sequences, and underscore the largely untapped potential of analyses of raw reads in molecular evolution studies. Equipped with an intuitive graphical interface, the framework enables large-scale and near-real-time monitoring of intra-host recombination, providing a new dimension for understanding genome-wide viral evolution.

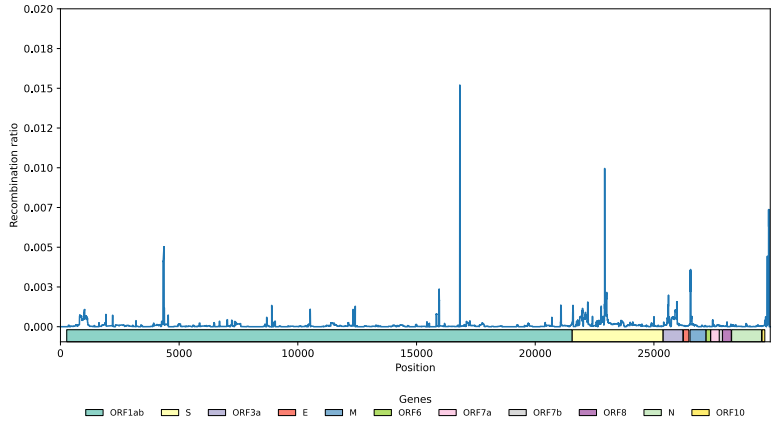

Figure 1. Genome-wide recombination profile of SARS-CoV-2 detected from around 150 000 public sequence reads.

LDGV was funded by a fellowship from Xunta de Galicia ED481A-2023/089, programa de axudas á etapa predoutoral da Xunta de Galicia (Consellería de Cultura, Educación, Formación Profesional e Universidades) cofinanciado pola Unión Europea no marco do Programa FSE+ Galicia 2021-2027. MA was supported by the Grant CNS2023-144363 funded by MICIU/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR.

Investigation of new conformational pathways in systems based on chiral Poly(phenylacetylene)s

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Stimuli-responsive chiral helical polymers usually bear a pendant group whose conformational composition can be altered by the presence of an external stimuli. This structural change at the pendant group led to a different spatial arrangement of the substituents at the chiral center that can result in a helix inversion, asymmetry amplification or helical excess depletion of the polymer. In this work, we show an unexplored conformational communication mechanism triggered by steric effects at the helical scaffold and where more than one bond is involved in a helix inversion process. Thus, by using different meta-substituted PPAs, we demonstrate how variations in the conformational composition at the chiral pendant by solvent polarity or metal ions, triggers a second conformational change at the linker used to connect the chiral pendant and the poly(phenylacetylene). Moreover, while variations at the conformational composition in the pendant does not affect to the helical sense of the PPA, the subsequent rotation at the linker triggered to release the steric hindrance generated in the PPA results in a final helix inversion process. Thus, this work opens a new scenario to create stimuli responsive polymers based on complex adaptive conformational communication mechanisms.

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Photothermal Effect in plasmonic nanostructures

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Gold nanorods (GNRs) are excellent candidates for hyperthermia applications due to their high chemical stability, ease of functionalization, low toxicity, and large extinction cross section. Moreover, their plasmonic bands can be precisely tuned by adjusting their aspect ratio (Figure1A), in the so-called biological windows, where light penetration in tissue is maximized.

Recent advances in synthetic techniques have made it possible to obtain intrinsically chiral gold nanorods (c-GNRs) by using achiral nanorods as seeds and introducing chiral thiolated molecules or co-surfactants as inducers of chirality[1]. These c-GNRs exhibit plasmonic modes that interact differently with right- and left-circularly polarized light (R- and L- CPL), a phenomenon known as circular dichroism (CD), wich is the asymetric extiction by the samples when irradiated with CPL of both handedness (Figure1B). This effect opens the door to use c-GNRs in photothermal therapies, employing CPL to achieve more selective heating.

In this context, we present the synthesis and characterization of c-GNRs using achiral GNRs as seeds, with a focus on enhancing their chiral optical response. We also evaluate the key parameters, i.e. the size and concentration of gold nanoparticles, influencing the heating efficiency of these nanoparticles, and analyze their photothermal effect under different irradiation conditions.

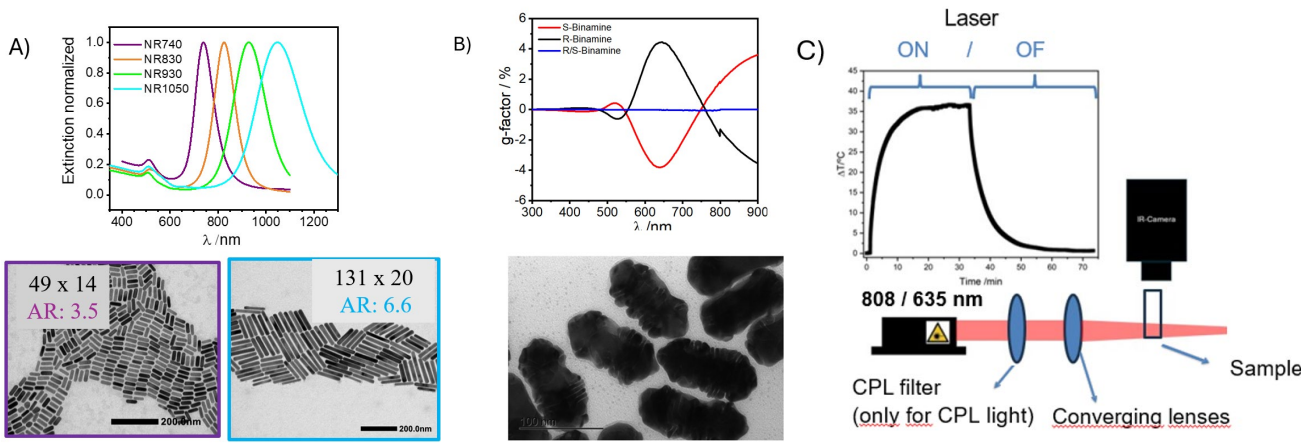

Figure 1. A) Extinction of GNRs of differenet aspect ratio with TEM images below(following the color leyends) B) g-factors of c-GNRs with TEM image below. C) Set-up for photothermal experiments.

The authors thanks the Xunta the Galicia for its financial support (ED481A 2024/224).

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In Quest for Polyenic Macrolactam JBIR-150 Identity: Stereoselective Synthesis of DP4+-Based Purported Structure and Biogenetically Inspired Positional Stereoisomers

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Polyenic macrolactam JBIR-150 (**1**) was recently isolated from the culture broth of *Streptomyces* sp. OPMA00071 on an Okinawan marine sediment. JBIR-150 (**1**) displayed cytotoxic activity against human malignant mesothelioma MESO-1 and human T-lymphoma Jurkat cells, with IC_{50} values of 2.3 and 0.90 μM, respectively, but weak cytotoxic effects against human ovarian adenocarcinoma SKOV-3 cells when tested at 100 μM.[1]

The total synthesis of polyenic macrolactam JBIR-150 with all-trans geometries, hydroxyl substituents located at C8 and C10 and 8*R*,10*R*,19*R* absolute configuration, the most likely stereoisomer predicted by DP4+ calculations, was completed. The construction of the conjugated triene units was based on a Suzuki-Miyaura cross-coupling of the corresponding alkenylboronates and alkenyliodides, and Horner-Wadsworth-Emmons (HWE) condensation reactions. Macrolactam formation promoted by HATU and DIPEA was best carried out by amine protecting group exchange and release of both reactive functional groups under basic conditions. Given the lack of correspondence of the ^{1}H- and ^{13}C-NMR data for the synthetic and purported JBIR-150, the hydroxyl groups of JBIR-150 were alternatively proposed to be located at the odd-numbered positions (C9 and C11) based on the general biogenesis of the family of polyenic macrolactams and the collinearity principle of their modular sequence. The C11-diastereoisomers of the biogenetically inspired 9,11-dihydroxy positional isomers (termed *iso*-JBIR-150) with formal *syn*- and *anti*-diol configurations was also completed. Much to our dismay, the NMR data of the synthetic diastereomeric polyenic macrolactams with either 9*S*,11*S*,19*R* or 9*S*,11*R*,19*R* absolute configuration did not match those of the natural product and, despite our efforts, the structural identity of JBIR-150 remains undetermined.

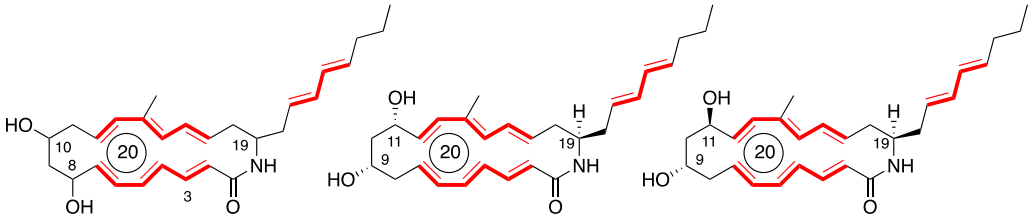

Figure 1. Structures of natural polyenic macrolactams JBIR-150, and alternative structures named iso-JBIR-150.

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Heterogeneous molecular evolution and diversity among genomic regions of emerging viruses

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Despite widespread vaccination efforts, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to pose significant questions regarding its future impact on public health. Indeed, other emerging viruses, such as the Crimean-Congo haemorrhagic fever virus (CCHFV), do not present yet a vaccine. In this regard, understanding the virus molecular evolution, particularly in protein-coding regions targeted by treatments, is fundamental. We analyzed a large number of SARS-CoV-2 and CCHFV genomes to assess the diversity, rates of evolution and molecular adaptation in these viruses. Overall, we found a wide variation of evolutionary patterns among genomic regions and over time, indicating a complex forecasting molecular evolution in these viruses, which could provide relevant information for treatments design, and remarks the importance of monitoring virus evolution.

This study was supported by the Grant CNS2023-144363 funded by MICIU/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR. LDGV was funded by «Programa de axudas á etapa predoutoral da Xunta de Galicia (Consellería de Cultura, Educación, Formación Profesional e Universidades) cofinanciado pola Unión Europea no marco do Programa FSE+ Galicia 2021-2027».

Impact of Synthetic Parameters on the Size and Morphology of COF-300

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Three-dimensional covalent organic frameworks (3D COFs) have recently garnered significant attention due to their highly ordered structures, inherent porosity, and outstanding thermal and chemical stability. Their interconnected channels and high surface area make them ideal candidates for applications such as energy storage, gas adsorption and separation, heterogeneous catalysis, and targeted drug delivery [1,2]. In this study, we focused on studying the influence of synthesis conditions on the size and morphology COF-300, a well-known 3D COF, with the aim of retaining its crystallinity and high surface area.

We systematically investigated the influence of key synthetic parameters, including solvents, reaction time, and temperature, on the resulting COF morphology and structural order. In addition, the influence of activation conditions was studied. Our findings revealed that reducing the reaction time to enable a faster synthesis, coupled with a lower reaction temperature and optimized activation procedures, not only preserved the crystallinity of COF-300 but also significantly decreased the particle size to the nanoscale range. This approach led to the formation of crystalline frameworks with uniform particle size distribution. Overall, our results suggest pathways for controlled engineering of 3D COFs, offering valuable insights for the development of advanced functional materials.

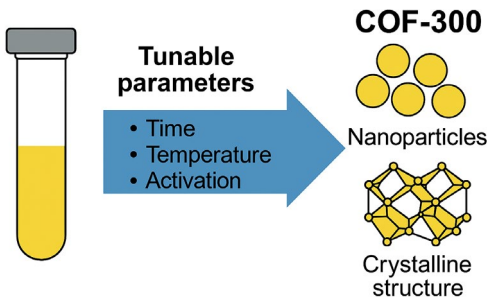

Figure 1. Tunable synthesis of COF-300 nanoparticles

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Microfluidic sample preparation for magnetic activated sorting of bacteria

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Magnetic-Activated Cell Sorting (MACS) is a widely used technique for isolation of specific cell populations based on surface markers, employing magnetic beads and column-based separation systems [1]. While effective, upstream sample preparation steps—such as incubation, washing, and labelling—are often labour-intensive and susceptible to variability [2]. Integrating on-chip modules for mixing, incubation, and washing within microfluidic systems can streamline the workflow and ensure consistent sample delivery to the MACS column. This integration is especially beneficial in clinical and research settings that demand high precision and scalability in cell isolation protocols.

Here, we present a microfluidic platform based on membrane filter for cell labelling and washing, which is designed to optimize sample preparation for MACS. This method is demonstrated using a mixture of engineered E. coli expressing specific nanobodies on their surface. The chip enables efficient incubation of the bacteria samples with biotinylated antigens and anti-biotin magnetic beads, facilitating the selective isolation of target bacterial cells.

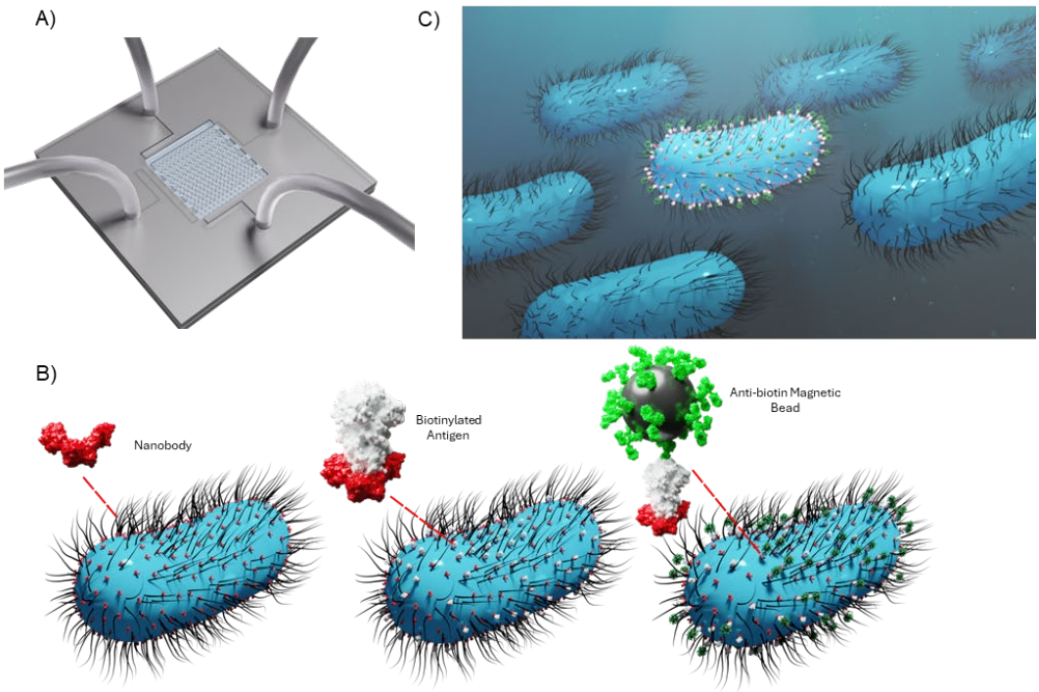

Figure 1. (a) Illustration of the microfluidic chip designed for on-chip sample preparation for Magnetic-Activated Cell Sorting (MACS). (b) Sequential steps of E. coli preparation including labelling with biotinylated antigen and anti-biotin magnetic beads (c) Illustration of the output flow showing the magnetically labelled target bacteria.

This work has been supported by funds from the EIC Pathfinder programme 2023 of the European Innovation Council (EIC) through ALADDIN project under grant agreement #101130574

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Green Extraction of Bioactive Compounds from *Castanea sativa* Mill Using Microwave-Assisted Autohydrolysis for Future Applications

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Chestnut shells, a by-product of *Castanea sativa* Mill widely cultivated in Europe, make up about 6–10% of the fruit's weight and are often discarded, despite being rich in polyphenols, lignin, and fermentable sugars [1,2]. Green extractions like microwave-assisted autohydrolysis (MAA) offer solvent-free, energy-efficient recovery of these compounds while preserving their activity [1,2]. However, there's still limited research on improving selectivity and yields from chestnut shells [3]. This study aimed to explore suitable conditions of MAA for extracting bioactive compounds followed by membrane separation for identifying the most active fractions for future applications.

Ground inner chestnut shells were treated using MAA with a solid–liquid ratio of 1:30, at 130 to 200 °C from 5 to 20 minutes. The best outcome (in terms of TPC and antioxidant activity) was achieved at 170 °C for 20 minutes. To improve efficiency, shorter times (down to 2.5 min) were tested at constant temperature, and the best results were maintained with 0.320 g GAE/g extract and 1.865 g Trolox equiv./g extract. Extracts were filtered through membranes ranging from 100 to 1 kDa. The 30–50 kDa and <1 kDa fractions showed the highest activity and were selected for further characterization (Table 1).

Table 1. Characterization of the original extract and selected fractions.

	Original extract	30-50 kDa	<1 kDa
Extraction yields (g dry content / g dry material)	28.04%	21,13%	0.66%
Total phenolics (TPC, g GAE /g dry extract)	0.1480± 0.0031	0.3100± 0.0044	0.6250± 0.0019
Condensed Tannins (Vanillin assay, mg (+)-catechin/ g dry extract)	0.1142± 0.0085	1.0948 ± 0.0554	0.0216± 0.0074
ABTS antiradical capacity (TEAC, g/g dry extract)	0.8014± 0.0079	1.3138± 0.0591	2.5045 ± 0.3766
DPPH antiradical capacity (DPPH, PI%: mg/mL)	67.62:0.001	61.13 : 0.0006	87.67 : 0.0009

The maximum phenolic purity was 310 mg GAE/g dry extract (or 6.55 g GAE/100 g dry material), highly dependent on the extraction conditions. Previous studies showed the maximum TPC content as 160.70 mg GAE/g dry matter [1] and 13.4 g GAE/100 g oven-dried shells (extracted with 2.5% Na2SO3 solution, [2]).

The <1 kDa fraction, although obtained in low yield, showed the highest antioxidant activity and phenolic content. On the other hand, the 30–50 kDa fraction had the highest tannin content and a much better yield. Compared to these, the original extract showed a more balanced but less specific profile, confirming that fractionation helps to selectively enrich target compounds depending on the intended application.

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A microfluidic device for on-chip isolation and recovery of extracellular vesicle-associated DNA

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Extracellular vesicles (EV) are membrane-bound vesicles with large heterogeneity in size and molecular composition [1]. This heterogeneity provides high diagnostic value, making EVs promising candidates for non-invasive biomarker detection and personalized diagnostic approaches [2]. However, the clinical adoption of EVs has not yet been achieved due to the lack of an efficient isolation and enrichment method that could be easily implemented into clinical routine. Currently, the most widespread method is differential ultracentrifugation, which is labor-intensive and time-consuming [3]. To overcome this challenge, microfluidics can be an advantageous alternative, reducing the size and cost of instrumentation, enabling automation and reducing required processing time. Furthermore, the clinical relevance of DNA in EVs (evDNA) has remained largely unexplored, despite the unique possibility to access the mutational landscape of the originating cells through their EVs.

To improve methods for studying evDNA and establish EVs as a standard-of-care, we have been developing a microfluidic platform for DNA extraction from EVs, which we coined “evDNA chip” presented here. The platform is composed of two parts, a herringbone mixer followed by an array of capture pillars. First, the mixer combines the sample (e.g., plasma, conditioned medium, saliva) with an EV aggregation agent, leading to the formation of EV-aggregates, which are then captured by the pillars in the next module. After capture EVs are washed to remove unbound material and lysed on-chip to recover the evDNA for downstream analyses.

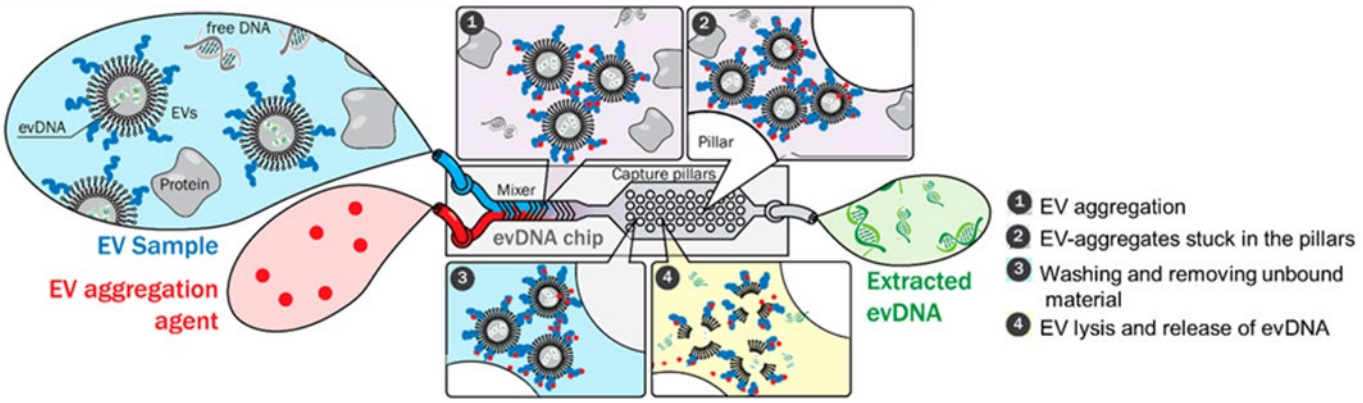

Figure 1. Concept of evDNA chip.

Acknowledgement: This work was supported by Health From Portugal (C630926586-00465198), co-funded by Component C5 – Capitalisation and Business Innovation under the Portuguese Resilience and Recovery Plan, through the NextGenerationEU Fund.

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Probing epithelial-mesenchymal transition in non-small cell lung cancer

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Metastases drive systemic dysfunction in multiple organ systems, contributing significantly to patient mortality [1]. During dissemination, cancer cells undergo phenotypic transitions that enable their survival, adaptation to secondary sites and initiation of metastatic outgrowth. In lung cancer, epithelial-mesenchymal transition (EMT) associated cellular plasticity facilitates organ-specific metastasis through mechanisms such as chemotherapy resistance [2] and collective invasion patterns [3]. However, the impact of EMT on the mechanical properties of cancer cells and subsequently their extravasation dynamics at secondary sites is poorly understood. Here, we demonstrate that induction of EMT in A549 or HCC827 non-small cell lung cancer cells, treated with 5 ng/mL TGF-β in DMEM medium with 2% FBS for one week, is accompanied by alterations in cell mechanical properties. Furthermore, such changes depend on the initial phenotypic state of the cell, thereby highlighting the heterogeneity of cancer cells within the primary tumour. Morphologically, cancer cells undergoing EMT acquire a spindle-like shape, in contrast to the round shape observed in non-treated cells, independently of cell type, (Fig. 1A). Experimental evidence from live-cell atomic force microscopy using Quantitative Imaging mode (cantilever spring constant of 0.25 N/m, tip radius of 30 nm, *z* length of 4000 nm, imaging area of 60 x 60 μm^2, 4096 data points per sample), at 37ºC, suggests the EMT increases the elastic modulus of both cells transitioning from epithelial to mesenchymal phenotype (Fig. 1B). Pixel-wise force-spectroscopy mapping at a maximum indentation force of 0.4 nN, reveals a stiffer nuclear region compared to the cytoplasm, likely providing mechanical protection to the nuclear contents during hematogenous dissemination. Ongoing studies are addressing the extravasation dynamics and invasiveness characteristics depending on their phenotype.

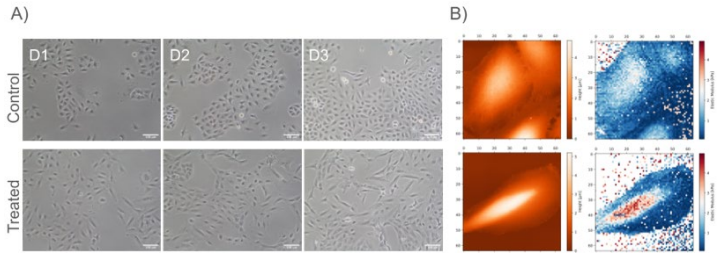

Figure 1. Characterisation of EMT in A549 cells during the initial 3 days of exposure to 5 ng/mL TGF-β . A) Cell morphology and B) live cell height (left) and stiffness maps (right) on the third day.

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Label-free SERS Biosensing of Saliva-based DNA Methylation for Oral Cancer Detection

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Early oral cancer (OC) detection is key for improving patient outcomes but remains challenging due to the lack of specific biomarkers and effective non-invasive screening tools. Aberrant DNA methylation occurs early in cancer and may be detected in liquid biopsies, constituting a valuable biomarker for cancer detection [1]. In addition, surface-enhanced Raman spectroscopy (SERS) stands out as a highly sensitive and rapid method for biomolecule sensing, making it a powerful tool for the early diagnosis of cancer [2]. This work aims to develop a SERS-based approach to assess specific gene methylation in saliva for OC detection. SERS substrates are synthesized by loading Au nanoparticles (Au NPs) on the surface of polyvinylidene fluoride (PVDF) membrane. Target DNA sequences will be amplified by PCR using methylation-independent primers on saliva samples form 30 OC patients and 30 healthy controls, and SERS spectra from PCR products will be acquired by using a Raman spectrometer. Further, multiple linear regression will be used to deconvolute the SERS spectra and calculate the methylation levels of the target genes. The development of a SERS-based methylation biosensor could enhance rapid, sensitive, and label-free detection of these methylation biomarkers, supporting its potential as a point-of-care diagnostic tool.

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Synthesis of different hydrophobic core nanoemulsions for cancer treatment

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Cancer is considered a major societal and public health issue, accounting for about 9.7 million deaths worldwide in 2022 [1]. A key factor contributing to this high mortality rate is the late stage at which many cancers are diagnosed, often after metastasis to distinct organs has occurred. Conventional and combination treatments often have several side effects and a poor quality of life for patients.

Photodynamic Therapy using photosensitizer-loaded nanoemulsions (NEs) has emerged as an alternative approach for selectively targeting cancer cells while minimizing systemic toxicity [2]. In this context, we propose the formulation of oil-in-water NEs using as hydrophobic photosensitizer verteporfin (VP), which is activated under near-infrared (NIR) light exposure, causing cell death [3].

Four different NEs were developed based on their fatty acid core composition: palmitoleic acid (POA), monounsaturated, and palmitic acid (PA), saturated. VP was successfully encapsulated at several concentrations, and encapsulation efficiency was determined through spectrophotometry.

Physicochemical characterization was performed utilizing: Transmission Electron Microscopy, Nanoparticle Tracking Analysis, and Dynamic Light Scattering, to evaluate particle concentration, size, polydispersity index, and surface charge.

In vitro assays in SKOV-3 (ovarian) and A549 (lung) cancer cell lines showed efficient VP-NEs internalization and light-dependent cytotoxicity upon NIR activation, implying their high therapeutic potential.

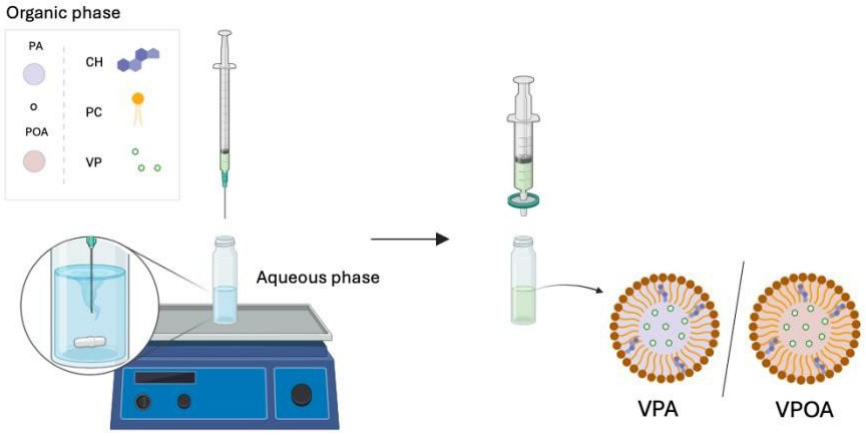

Figure 1. Formulation procedure of VP-NEs. PA, palmitic acid; POA, palmitoleic acid; CH, cholesterol; PC, phosphatidylcholine; VP, verteporfin.

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Unraveling Breast Cancer Heterogeneity: Deformability-based Microfluidic Sorting and Bioassay-based Functional Analysis

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The metastatic potential of cancer cells is closely linked to their mechanical properties, which evolve with cancer progression and heterogeneity. Small and large tumor cells play different roles, with smaller cells exhibiting higher proliferative potential and initiating new tumors, while larger cells may be more differentiated or adapted to specific microenvironments [1]. However, robust tools are needed to sort and analyze these subpopulations effectively. Here, we present a deterministic lateral displacement (DLD)-based microfluidic device for sorting MDA-MB-231 breast cancer cells by size. A DLD device with three inlets and outlets was utilized to fractionate breast cancer cells into subpopulations: small and large. The sorting efficiency is validated using inverted microscopy and post-sorting behaviour is analysed through long-term culturing and functional assays.

Biological differences between subpopulations were assessed through proliferation to monitor growth rates over one week and migration assays to examine motility differences across subpopulations. Over a week-long period, all sorted subpopulations continued to proliferate, with small cells displaying a significantly higher motility compared to larger cells and unsorted controls, reinforcing the idea that smaller cells exhibit enhanced metastatic potential. These findings align with previous reports highlighting the role of cytoskeletal adaptations in invasion dynamics. In addition to that, holographic microscopy provided real-time tracking of cellular dynamics. Our study highlights DLD-based microfluidics as a high-throughput, label-free approach for studying cancer heterogeneity. Ongoing work focuses on generating 3D spheroid models from sorted subpopulations to explore tumor progression, drug resistance, and invasion mechanisms, ultimately advancing targeted therapies for aggressive breast cancer. The next step is to integrate deformability-based sorting of breast cancer cell lines at varying metastatic stages, enabling more precise characterization of biomechanical phenotypes linked to cancer progression.

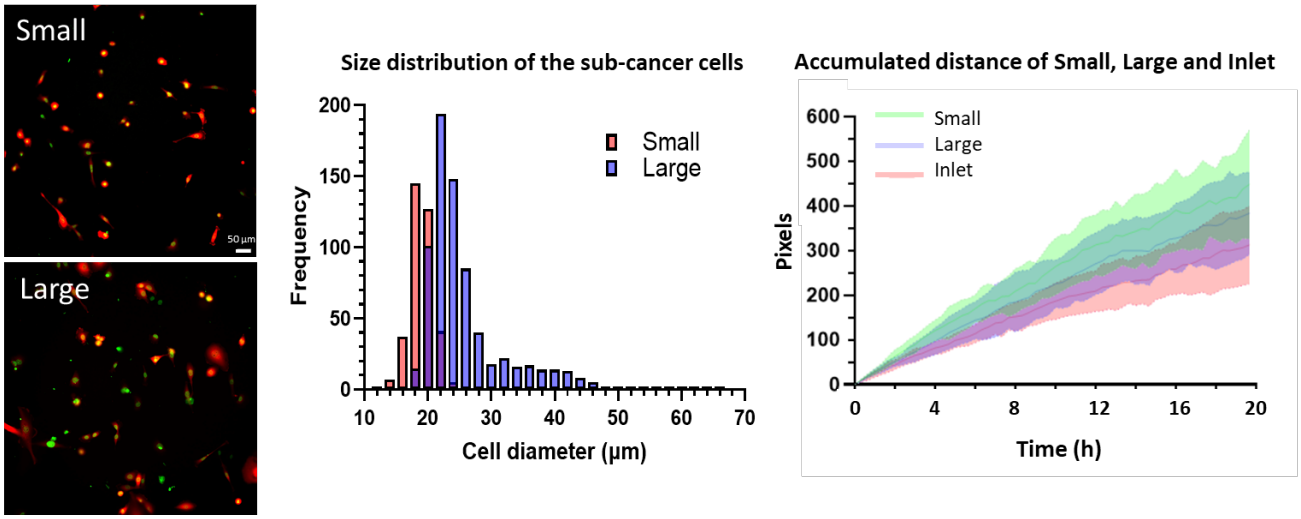

Figure 1. Microfluidic sorting followed by size analysis and migration assay

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POSTER SESSION

II

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EV-based mRNA therapy as a novel approach for TNBC

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Breast cancer (BC) is the most diagnosed cancer worldwide. Among its subtypes, **triple-negative breast cancer (TNBC)** is characterized by high aggressiveness and poor prognosis, currently lacking effective targeted therapies. Among potential treatment strategies, **PARP inhibitors (PARPi)** and **immunotherapy** offer new hope for TNBC patients, particularly those with **BRCA1/2 mutations**. Despite initial success, preclinical and clinical studies have shown limited efficacy and the emergence of resistance mechanisms, underscoring the need to identify novel functional targets and combination strategies to enhance therapeutic outcomes. We have identified **connexin 43 (Cx43)** as a promising biological target with anti-tumor activity. Cx43 enhances the efficacy of PARPi and immunotherapies by interfering with DNA damage response pathways and restoring anti-tumor immunity. Based on these preliminary findings, our objective is to explore a new strategy to study the therapeutic efficacy of restoring Cx43 expression via **mRNA therapy**, using **extracellular vesicles (EVs)** as delivery systems. As a first step, we generated a TNBC murine cell line model by introducing a **BRCA1 mutation**. EVs enriched in Cx43 were obtained from genetically engineered **HEK293 cells**. EV size and characteristics were analyzed using **nanoparticle tracking analysis (NTA)**, **Western blot**, and **flow cytometry** to confirm uptake. These EVs not only showed high levels of Cx43 protein but also carried the corresponding mRNA. Initial **in vitro assays** suggest that this strategy has several advantages: it enhances anti-tumor immunity and shows promising results in overcoming resistance when combined with PARPi. Together, these results support the potential of this approach as a novel therapeutic strategy for TNBC patients.

Beyond CTC clinical value: generation of low-cell-number aggregates for breast cancer metastasis studies

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Circulating tumor cells (CTCs) play a pivotal role in metastasis – the underlying cause of cancer-related fatalities – making them interesting biomarkers for early detection and relapse prevention. The clinical value of CTC detection has been established [1], however, their potential beyond the clinical setting, as a tool to identify metastasis competent cells, is still not explored. Evidence shows that CTCs share common genetic profiles with active tumors (primary or metastatic), yet most cancer research focuses on studying these tissues directly. As a result, many aspects of the metastatic cascade remain underexplored, in part due to the limited availability of methods and models to investigate CTCs. Organ-on-a-chip technology has gained significant attention due to the higher level of biomimicry and complexity compared to the traditional model platforms. Within a device we can reproduce important cell to cell and cell to extracellular matrix interactions while providing vascular perfusion making it ideal to replicate the in vivo scenario. As such, combining patient-derived CTCs within these microfluidic platforms can contribute to a better understanding of metastasis. However, the scarcity of CTCs in peripheral blood (typically about 1-10 CTC per mL) makes their expansion challenging. To address this, we developed proliferation methods using MCF-7, SKBR3 and MDA-MB-231 cell lines, considering the existing breast cancer subtypes. These cells, served as models to generate aggregates with a low starting number of cells, mirroring the conditions typically encountered in a liquid biopsy sample from a patient. With the conditions provided, we were able to develop cell aggregates starting with an initial cell number of 25 cells. The viability was monitored over 12 days of culture using a Live/Dead™ assay plus CellTiter Glo 3D and yielded positive results with continuous proliferation and good viability for the 3 different subtypes. To increase the complexity and relevance, stroma cells were added in a 1:3 ratio to the aggregates. The co-culture of cancer cells with human mammary fibroblasts seem to aid in the fast and more robust/compact generation of the aggregates, with good viability. Future experiments will include applying the methods in patient samples and subsequently transferring the CTC aggregates into a tumor-on-a-chip device to study key events of tumor progression, such as intravasation and metastasis.

We acknowledge the financial support from Fundação para a Ciência e Tecnologia (FCT) for the PhD scholarship 2022.14120.BDANA. This work was also supported by PROMISE project funded through the Caixa Health Research program from La Caixa Foundation (HR20-00637); Health from Portugal (C630926586-00465198), supported by Component C5 – Capitalisation and Business Innovation, under the Portuguese Resilience and Recovery Plan, through the NextGenerationEU Fund; and financed by project IBEROS+ (007, 2_IBEROS_MAIS_1_E, Interreg-POCTEP 2021-2027).

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Development of Microfluidic Fluorescence-Activated Cell Sorter (FACS) Platform for Low Fluorescence Samples

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The ability to sort cells based on their specific markers has become central to modern biological and biomedical research, enabling researchers to separate complex heterogeneous cell populations. Fluorescence-activated cell sorting (FACS) is a common active sorting technique allowing separation of cells based on their individual highly detailed characterization.

Integration of FACS with microfluidic can add many different functionalities to the analytical process provided by lab on a chip technology for sensing, upstream and downstream processing and automation, increasing versatility, while reducing required sample volumes and shear-induced damages and size of the instrumentation. However, challenges remain in achieving throughput comparable to conventional commercial FACS machines. Current microfluidic FACS platforms for mammalian cells achieve sorting rates of ~8,000 cells per second [1], while bacterial sorting lags further behind at ~2,777 events per second [2], underscoring the need for optimization of microfluidic architectures and detection systems for bacteria.

Here we present a novel microfluidic FACS platform to addresses these limitations by integrating a silicon photomultiplier (SiPM) detector, a high-sensitivity, yet compact and affordable single photon-counting device previously unexplored in FACS applications that can outperforms conventional photomultiplier tubes (PMTs) and avalanche photodiodes (APDs) in detecting weak fluorescence signals, such as those from low-abundance markers at a high processing rate.

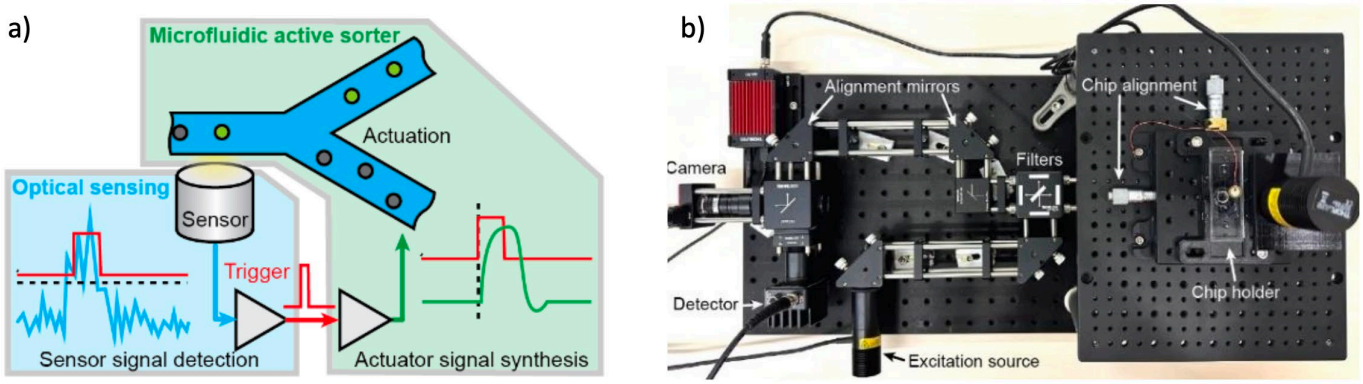

Figure 1a. Schematic diagram of a microfluidic cell sorter based on fluorescence detection and b) optical setup for microfluidic fluorescence detection. The system includes a camera, detector, excitation source, alignment mirrors, filters, and a chip holder

Acknowledgements:
This work has been supported by funds from the EIC Pathfinder programme 2023 of the European Innovation Council (EIC) through ALADDIN project under grant agreement

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Towards the implementation of a Pseudomonas aeruginosa Quorum Sensing biosensor in a standard CubeSat satellite space mission

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The bacteriological Intercommunication eXperiment in Orbit (BIXO) is a student-led initiative that aims to investigate the impact of prolonged exposure to radiation and microgravity in a Low Earth Orbit environment on bacterial Quorum Sensing (QS) [1]. The BIXO mission encompasses a 2U CubeSat satellite integrating both the spacecraft platform and the biological payload. The CubeSat standard is a specification for small, standardized satellites that facilitates cost-effective access to space [3]. We focus the biological payload on the human opportunistic pathogen Pseudomonas aeruginosa, a widely used model for studying QS and biofilm formation (PMID: 36258070). To monitor QS in the CubeSat satellite, the bacteria will be lyophilized and enclosed within microfluidic cards housed within a pressurized chamber. Once in space, bacterial communication will be evaluated by fluorescence spectroscopy of P. aeruginosa cells bearing the pAC37 plasmid, which expresses a green fluorescent protein (GFP) as a proxy of QS [4]. Herein, we report preliminary data regarding the lyophilization and the evaluation of QS in P. aeruginosa under laboratory conditions. The ultimate goal of this study is to contribute to the understanding of astrobiology, particularly how microorganisms respond to harsh space conditions in miniaturized space missions.

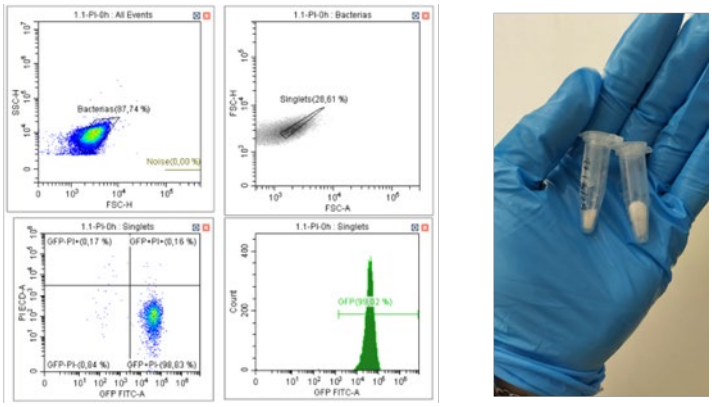

***Figure 1. A** - Representative flow cytometry graphs of P. aeruginosa bearing the pAC37 plasmid expressing GFP. **B** – Lyophiles of plasmid-transformed P. aeruginosa obtained with the Reagent 18 cryoprotectant.*

Acknowledgement of funding: We thank CINBIO, especially FunNanoBio group for funding support. (Reference: CNS2023-145718)

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TRANSANNULAR (TADA) BIS-[4+2]-CYCLOADDITION OF THE MACRODIOLIDE FOR THE STEREOSELECTIVE SYNTHESIS OF THE OCTAHYDRONAPHTHALENE CORE OF POLYENIC MACROLACTAM SAGAMILACTAM

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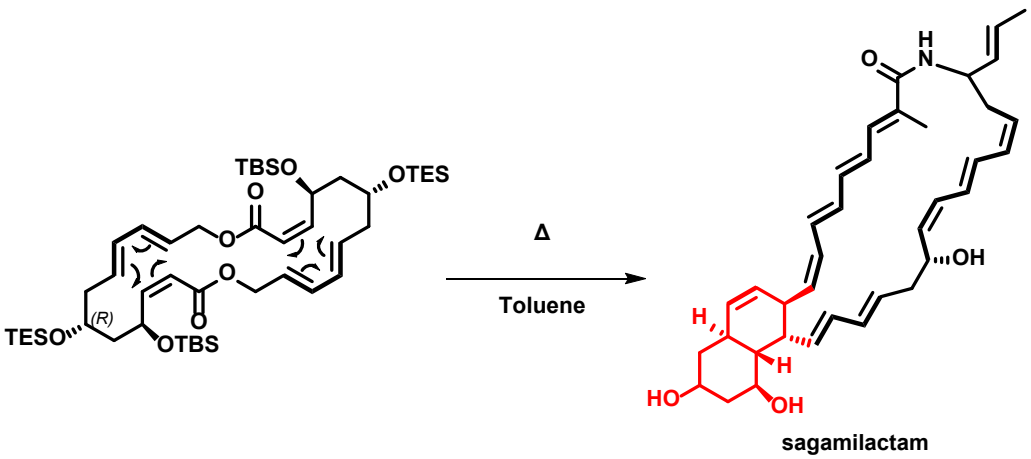
Keywords: transannular Diels-Alder, stereoselective synthesis, sagamilactam

Polyenic macrolactam sagamilactam was isolated in 2016 after bioassay-guided fractionation of the culture broth of Actinomadura sp. K13-0306 bacteria present in a soil sample collected in Kanagawa Prefecture, Japan, and exhibited potent activity (IC50 = 0.14 + 0.06 μg/mL or 0.25 + 0.11 μM) against the parasitic protozoan Trypanosoma brucei GUTat 3.1 strain.[1]

The strategy for the synthesis of the octahydronaphthalene core of natural macrolide sagamilactam has unintentionally evolved from the acyclic intramolecular (IMDA) to the transannular (TADA) Diels-Alder reaction. The Lewis acid-promoted IMDA of protected 2Z,8E,10E-4,6,12-trihydroxy-2,8,10-decatrienal model with a diol of 4,6-anti relative configuration, as proposed by DP4+-based computational studies, afforded the cis-octahydronaphthalene diastereomer through the Re-endo approach. By contrast, the 26-membered macrodiolide generated, under thermal reaction conditions, the trans-octahydronaphthalene by a double TADA reaction along the desired Si-exo orientation. In addition to the published findings,[2] we will describe our progress on the TADA reaction of stereoisomers and geometric isomers of the macrodiolide.



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Mechanisms of saliency coding in the SNc/VTA

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Dopaminergic neurons located in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) are triggered by salient events. Saliency is the ability of a stimulus to capture our attention, showing the capacity to stand out from other stimuli as potentially relevant, based on physical features, as speed, size or contrast. Rapid detection of important events in our surrounding is critical for survival. The SNc/VTA modulate the basal ganglia, a group of subcortical nuclei well conserved through vertebrate evolution, from lamprey, oldest extant vertebrate, to mammals. The SNc also provides direct dopaminergic projections to brainstem motor centres, including the optic tectum/superior colliculus (OT). We have previously shown that the OT projects to the SNc (Pérez-Fernández et al., 2014, 2017). Moreover, we also observed that activity in the SNc increased in parallel with increasing speed, an aspect involved in visual saliency, suggesting that the salience responses in the SNc may be originated in the OT, as has been suggested in mammals. However, the mechanisms by which saliency is coded and transmitted to the SNc are still unknown. For this aim, we used different visual paradigms, applying visual stimulation with screens, using a lamprey eye-brain preparation, to analyse how activity in the SNc changes in response to individual properties involved in saliency. Using pharmacology and lesioning different brain areas we analysed the neural connectivity and how these different areas are involved in salient responses in the SNc.

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Coestimation of substitution and recombination rates in protein sequences accounting for structural constraints

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Mutation and recombination are fundamental evolutionary processes, upon which selection operates, to produce the observed diversity in nucleotide and amino acid sequences. Although nucleotide sequences have been widely studied, the estimation of the recombination rate in protein sequences has been weakly explored. Indeed, commonly used evolutionary analyses of protein sequences ignore evolutionary constraints arising from the protein structure, which may cause selective pressures. Extending our previous works [1], we present the design of a method to coestimate population substitution and recombination rates from protein sequences, under structurally constrained substitution models of protein evolution [2], through the approximate Bayesian computation approach, which does not require likelihood calculations. We consider that substitution and recombination events can be characterized by summary statistics based on constraints from the protein structure, since it is known that some of these events can affect the protein folding stability [3]. We will apply the method to viral protein sequences and the findings will be compared with traditional estimation methods that ignore selection on the protein structure.

This work was supported by the Project PID2023-151032NB-C22 funded by MICIU/AEI/10.13039/501100011033 and by FEDER, UE.

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Formulation of Bioactive Microparticles from *Undaria pinnatifida* brown seaweed extracts obtained by ultrasound-assisted extraction

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Brown algae are high-value macroalgae that have attracted growing industrial interest due to their wide range of applications. In this project, Undaria pinnatifida (Up) was used as raw material, notable for its content of polysaccharides with biotechnological interest, such as laminarin (antioxidant and immunomodulatory properties), fucoidan (anticancer, antioxidant, and anticoagulant activities), and alginate (rheological properties). To sustainably extract these bioactive fractions, green extraction technologies like pressurized hot water, microwave, or ultrasound can be employed, avoiding the use of organic solvents reducing environmental impact [1,2,3].

In this study, the extracts were obtained by ultrasound-assisted extraction technology using distilled water as solvent. The extraction conditions included time treatments from 30 minutes to 3 hours, a solid-to-liquid ratio of 1:20 (w/w), a temperature of 40 °C, a frequency of 80 Hz, and 100% power. The extracts were characterized in terms of phloroglucinol content, antioxidant activity (measured as TEAC value), and oligosaccharide type and content.

The extract obtained after 3 hours of sonication showed the highest values in antioxidant capacity, sulfate, and protein content. However, no significant differences were observed in the results when compared with the obtained after 2 hours; therefore, the 2-hour extract was selected for further studies, allowing a reduction in energy, time, and resource consumption without compromising the efficacy. Regarding phloroglucinol content, the highest value was observed after 1 hour of sonication, being the difference with the extract obtained after 2-hour extract minimal. The slight decrease in phloroglucinol with longer sonication times may be attributed to compound degradation, further supporting the suitability of a shorter treatment duration.

Once the extract was selected, microparticles were formulated using commercial alginate. Representative images were obtained to assess the particle size distribution profile and morphological properties of the microparticles.

The confirmation of the bioactive potential of these microparticles from the brown seaweed Undaria pinnatifida could open new applications in different fields in biomedical terms.

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Fluorescently Labelled Extracellular Vesicles for Imaging and Analysis of Tumor Cell Communication

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Liquid biopsies are emerging as powerful, non-invasive tools for the discovery of novel biomarkers in cancer research (1). Within this field, extracellular vesicles (EVs) are gaining particular interest (2). Being actively released by tumor cells, and encapsulating bioactive cargo (DNA, RNA, proteins), they give valuable information about tumor phenotype and metastatic potential (2). Classical EVs characterization methods, like nanoparticle tracking analysis (NTA), rely on light scattering, which cannot distinguish between cell-derived EVs and other particle-like structures. EVs staining could aid this process, but typical dyes (e.g. PKH67) form self-dye micelles, thus interfering with EVs marking. Therefore, there is a critical need for robust methods to label and image EVs, not only for tracking during sample processing protocols, but also to elucidate their roles in cell-cell communication processes.

Here we propose two different approaches to generate fluorescently labelled EVs. The first one involves labelling the cancer cells with membrane permeable fluorescent dyes (e.g. calcein), which are subsequently incorporated into the EVs. The second one employs cells transfection with plasmid DNA encoding a fluorescently tagged EV membrane marker (e.g. CD63), leading to the production of inherently fluorescent EVs. EVs obtained from both approaches will be characterized using confocal imaging, total internal reflection fluorescence (TIRF) microscopy, and NTA.

With the development of fluorescent EVs we will enhance their visualization in microfluidic-based isolation techniques and allow real-time monitoring of EVs-mediated cell communication in advanced *in vitro* models such as organ on-a-chip systems.

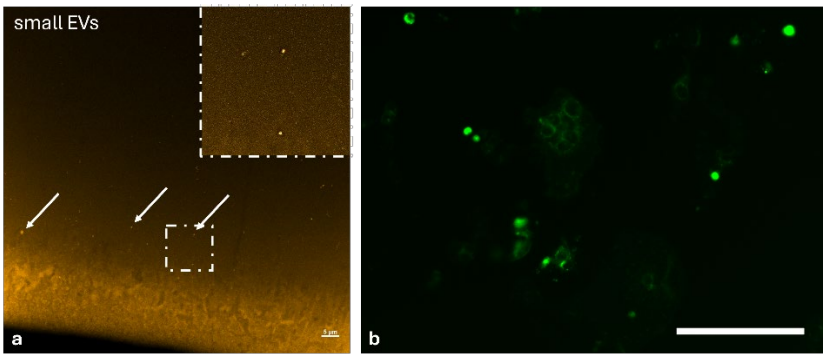

Figure 1. Preliminary results of the chosen approaches to generate fluorescent EVs, with cell-permeant fluorescent dyes (a, scale bar 5 µm) and with CD63-GFP plasmid DNA transfection (b, scale bar 150 µm).

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Extraction of bioactive compounds from Laminaria ochroleuca by pressurized hot water extraction as ecofriendly technology

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Fucoidan is a sulphated polysaccharide composed of fucose, glucose, other oligosaccharides and bioactive compounds. The main source of this compound are brown algae. The importance of this polysaccharide resides in its numerous biological activities as antioxidant, anti-inflammatory, antiviral and antitumoral properties [1].

The extraction of this compound, while maintaining these properties, is particularly interesting. With this purpose, in recent years several non-conventional extraction methods has been evaluated, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE) and sequential of them. In this study, pressurised hot water extraction (PHWE) has been proposed for the extraction of fucoidan from edible *Laminaria ochroleuca*, since it presents characteristics that make it a relatively simple green method, where using water as a solvent, good yields and high quality extract are achieved [2]. Furthermore, this seaweed is relatively common along the Atlantic coast, and the fact that it is edible makes it even more interesting from the perspective of its use in various areas.

Based on previous studies [2], the operating conditions are set at a solid-liquid ratio of 1:30 (w/w) and temperatures of around 160 ± 10 °C using non-isothermal conditions. Based on the characteristics of the extract in terms of phloroglucinol, sulfated content, antioxidant activity as TEAC value, and the oligosaccharide content the extract selected was obtained at 150 °C.

With this data and the information provided in previous studies on the bioactive potential of fucoidans [3], it would be interesting to develop the synthesis of microparticles with this extract, which could be applied to food, cosmetic or medicine fields.

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Droplet-microfluidics: versatile platforms supporting the analysis and functional studies of single cells

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The manipulation and analysis of cells at a single level is crucial to explore several fundamental biological processes and uncover variations among individual cells [1]. An attractive branch of microfluidics field, microdroplet-based microfluidics systems offer unique characteristics such as an isolated microenvironment for cells, preventing cross-contamination and enabling the concentration of molecules released by the individual cells as well as functional proliferation studies [2]. However, to apply mcirodroplets technology to single cells studies is crucial a deep control of microdroplets operations in within the microfluidic channels.

In this work we have optimised a series of microdroplet modules that allow in series the encapsulation and immobilisation (incubation or parking) of single-cell containing droplets. For single cell encapsulation we designed and fabricated flow-focusing devices with a geometry of (200 μm width by 100 μm height), leading to droplets of a diameter of approx. 120 μm. For this droplet diameter, the optimal concentration of cells to achieve the sought single cell encapsulation was found to be 2 million cells/mL. However, it is worth noting that in a context of analysis of rare cells (i.e. circulating tumour cells in cancer), there will be most of the droplets empty, while a small portion will contain the single cell of interest. Hence, after droplet generation there is a need of selecting the droplets of interest, using either a passive or an active sorting strategy. The former technique relies on the geometry and size of the microchannel, whereas the latter approach employs additional actuators to control droplet formation, encapsulation and sorting [3]. In both strategies, we aimed at producing droplets with a suitable size to accommodate and concede cell growth and proliferation as a first-proof-of concept of the technology. Additionally, for the microdroplet generation process we will also employ an adipose extracellular matrix that undergoes gelation after few minutes, creating a solid template scaffold. The protocol has been refined to enable the oil exchange into cell culture medium following droplet trapping and extracellular matrix gelation time, and to keep the system in continuous flow at 100 μL/h, thus facilitating the supply of nutrients for cell growth. Additionally, we also optimized a method to maintain the system closed during image acquisition and to prevent air from infiltrating the microfluidic device. As future work, droplets with single cells will be monitored over days to evaluate cell growth and proliferation [4].

This work is supported by the 3DSecret project, funded by the EU under the program HORIZON-EIC-2022-PATHFINDEROPEN-01-01 (ga 101099066) and by the UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee (ga 10063360)

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Potential role of TREK-like channels on cardiac pathologies against mechanical and temperature stimuli: an in vitro study

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The intrinsic cardiac nervous system, a complex circuit composed by the intracardiac ganglia (ICG), serves as the heart's final integration center, regulating local cardiac performance. ICG neurons express functional TREK channels, two-pore potassium channels (K2P) responsible for the maintenance of neuronal resting membrane potential and excitability. This subfamily responds to various physiological, chemical and physical stimuli, including mechanical stress, temperature and acidification, indicating their potential role in feedback in the heart. Here we investigated on primary neuronal culture of mice how ICG neurons respond to mechanical and temperature stimulation and their contribution to the maintenance of the resting potential. We discovered the under a mechanical distension ICG neurons response a decrease ICG neurons excitability, attributed to the activation of TREK-like channels, suggesting that mechanical stimulation influences these channels ultimately impacting neuronal activity. Regarding to the temperature stimuli, we observed that a rise in temperature decreases ICG neuronal excitability due to the activation of TREK like channels. Our findings indicate a possible neuroprotective role of TREK channels in the ICG during mechanical stress conditions such as ischemia and their potential role as targets for certain cardiac pathologies associated to body temperature changes, such as cardiac fibrillation and arrhythmia.

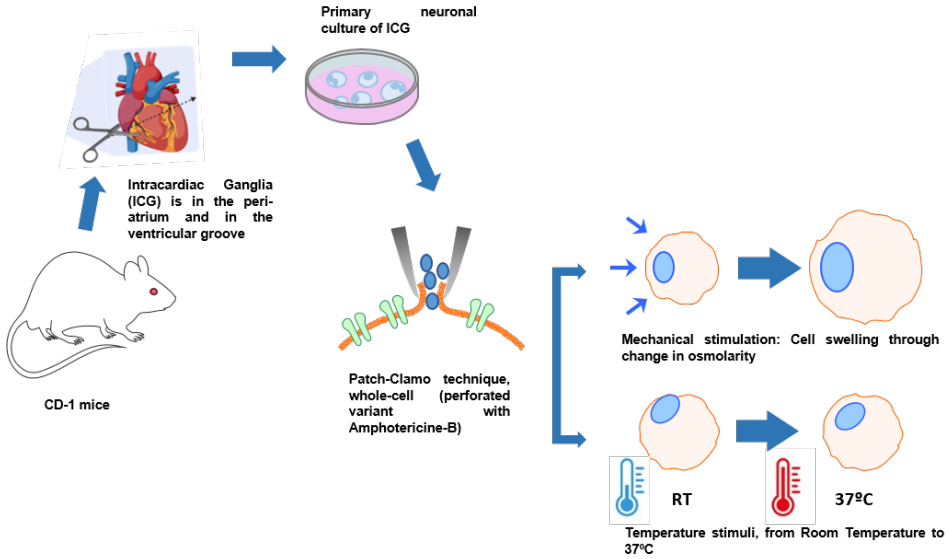

Figure 1. Intracardiac primary neuronal culture: after 24h of incubation these cells are used for the patch clamp technique (whole-cell perforated variant) and two experiments are performed. With an hipoosmotic solution we evoked a change in the volume of the cell imitating a mechanical distention triggering TREK channels. On the other side we performed temperature experiments rising from room temperature (RT) to 37ºC to activated the TREK channels. In both experiments we observed a change in neuronal excitability.

Recovering DNA using a chitosan functionalized microfluidic device

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Cell-free DNA (cfDNA) and extracellular vesicle DNA (evDNA) are promising biomarkers in modern biomedicine, carrying genetic signatures of diseases such as cancer, neurological disorders, and infections, allowing for early detections and monitoring[1]. While being primarily obtained from minimally invasive liquid biopsies, their low abundance, susceptibility to degradation and complex isolation requirements pose significant challenges for their clinical implementation. Microfluidics holds the potential to solve this, providing systems with high sensitivity, rapid workflow and automation capabilities. Historically, DNA capture has been performed using solid phase extraction (SPE), which leverages functionalized surfaces such as silica beads, membranes, or chitosan-coated surfaces, to bind DNA from biological samples. When combined with the confined, high-surface-area environment of a microfluidic device, SPE enhances DNA capture efficiency by exploiting reversible interactions between DNA and the solid phase, often mediated by chaotropic agents or pH adjustments [2]. Our proposed method uses arrays of micropillars functionalized with pH-responsive chitosan, exploiting chitosan's unique charge-switching properties for efficient and reversible DNA binding. We explored and contrasted the capture of short salmon DNA fragments (77-160 base pairs), emblematic of circulating cell-free DNA, with the robust λDNA (48k base pairs), a proxy for not only genomic DNA but also for evDNA (typically between 1k-10k base pairs [3,4]). The results not only demonstrate high efficiency in DNA capture but also highlight the possible synergies between distinct chitosan formulations and diverse DNA types. We also report on an evolving DNA recovery process, achieving 40-50% elution from chitosan functionalized pillars via pH-mediated release. On-going work is focused on refining strategies to disentangle persistent DNA-chitosan interactions for optimized recovery.

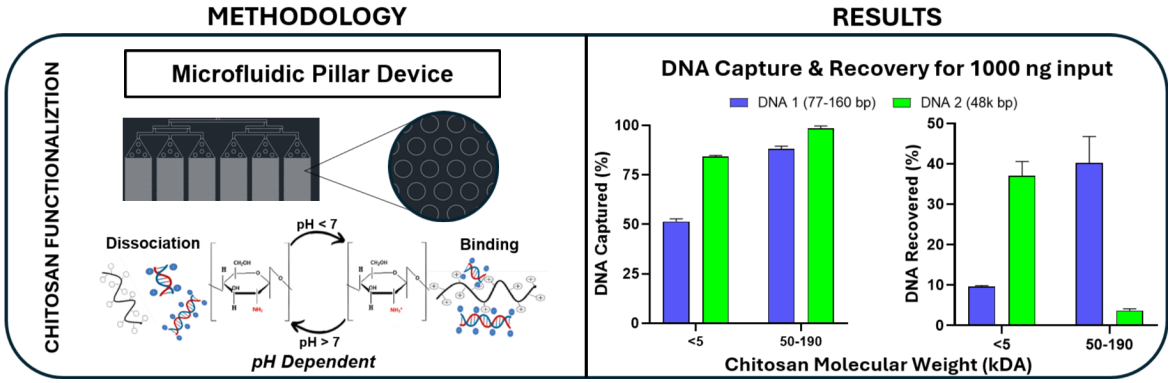

Figure 1. Schematic representation of the methodology (left) for the SPE chitosan functionalized microfluidic pillar device and results (right) obtained for DNA capture and recovery for different types of DNA and chitosan.

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New tools to characterize B cell mediated immune response in non-mammal vertebrates

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Turbot (*Scophthalmus maximus*) is an important species in aquaculture, but infectious diseases remain a major challenge, making the study of immune responses crucial. However, the lack of specific reagents limits research progress. Advances in genomics and transcriptomics offer valuable tools for studying immune responses. Despite these advancements, there is still limited understanding of the mechanisms behind B and/or T cell-mediated immune responses. In teleosts, like mammals, adaptive immunity relies on B cells expressing diverse immunoglobulins (IG). High-throughput sequencing (HTS) is a valuable method for analyzing the IG repertoire. Aquaculture species like salmonids benefit from HTS, which improves our understanding of immune responses to pathogens and vaccines.

In this context, identifying the best protocol for obtaining high-quality libraries is essential. Our study compares two protocols: one using specific oligos targeting V subgroups and another based on 5'RACE with oligos targeting the constant region. We also compare two turbot genomes, the Atlantic fScoMax and the Asian ASM2237912v1, as the Nomenclature Committee requires genomes to have published sequence files (SRA). This comparison aims to identify allelic differences and determine whether IGHV gene subgroups cluster similarly in both ecotypes. This study highlights the need for improved methodologies and genomic tools to advance immune research in turbot, which will enhance disease resistance in aquaculture.

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Mechanical-Based Cell Sorting Using Multi-Dc DLD Microfluidic Devices

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The efficient and selective separation of micron-scale particles represents a fundamental challenge across various biomedical fields, from early diagnostics to monitoring tumour progression, or even for sample preparation for molecular analysis. In this context, Deterministic Lateral Displacement (DLD) devices emerged as a powerful microfluidic technology, capable of performing label-free separations based not only on particle size, but also on shape and deformability [1]. The operating principle relies on the controlled interaction between suspended particles and an ordered array of micropillars, which induces flow deviations depending on the physical properties of the sample (Fig.1a)[2-4].

In this work, a series of DLD devices with five different critical thresholds (Dc: 10 µm, 12.5 µm, 15 µm, 17.5 µm, and 20 µm) were developed and tested (Fig.1b). These devices were optimized to explore the behavior of both rigid and soft particles, as well as cancer cells characterized by varying mechanical stiffness. In particular, the study focuses on two breast cancer cell lines: MCF7, which are stiffer and exhibit a less invasive phenotype, and MDA-MB-231, which are more deformable and highly metastatic. Comparing these cell lines allows for an assessment of how biomechanical properties influence interaction with the DLD pillar array, resulting in different trajectories beyond size-based separation.

Preliminary results with the Device having Dc:10 µm indicate that, at the outlet corresponding to particles with diameters > Dc, separation was 99% efficient (Fig.1c). However, at the outlet associated with particles < Dc, a mixed distribution was observed, consisting of 70% of 8 µm particles and 30% 15 µm particles. These experimental findings confirm the DLD device capability to achieve effective separation for particles significantly larger than the critical diameter. This work provides valuable insights for optimizing both the operational conditions and the design of DLD devices, with the goal of improving separation resolution near the critical size—particularly in view of applications involving human cells, which are heterogeneous in size and mechanical properties.

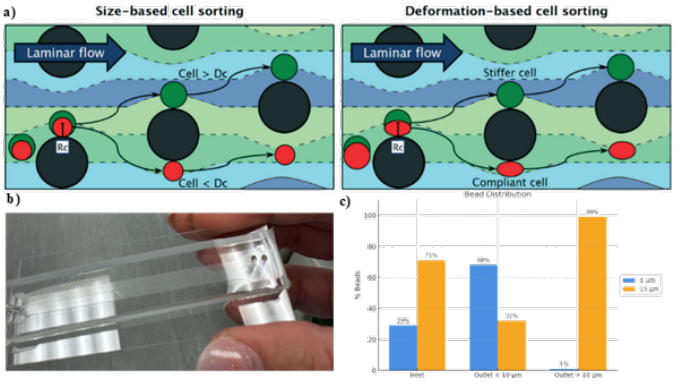

Figure 1. (a) *Schematic representation of particle selection through deterministic lateral displacement based on differences in particle size (left) and deformability (right) [1].* (b) *PDMS casting of the device.* (c) *Fraction of 8 µm beads (blue bars) and 15 µm beads (orange bars) collected at the two outlets of the device shown in (b), as a function of size at a flow rate of 5 µl/min.4*

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Microwave-assisted extraction of bioactive compounds from green algae

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In recent years, due to the constant global population growth and the consumers demand for natural derived ingredient, there has been a high demand of bioactive compounds for food, nutraceuticals and cosmetics production. For this reason, alternative foods with a high nutritional value and obtained through sustainable processes have been sought[1]. Green macroalgae represent a promising resource that can be either cultivated or collected from blooms, which are events occurring with higher frequency due to human activities and global warming. The major components of these seaweeds are polysaccharides, but they also contain bioactive compounds that are could be used in the food industry or can be incorporated into foods as active and functional ingredients[2]. Previous studies of the group have confirmed the potential of microwave assistance for the successful extraction of ulvans and the importance of the salt content of the medium for their recovery[3], but valorization of other valuable fractions is desirable.

The objective of the present study is to optimize the extraction of the bioactive compounds present in the green macroalgae *Ulva* spp using a microwave-assisted technology. Different temperatures were evaluated in the extraction process, using a biomass/water ratio of 1:30 (w:w) with extraction time of 5 min and cooling temperature of 55 °C. Total Phenolic Compounds, TEAC, FRAP, and DPPH were spectrophotometrically determined. Minerals, sulfate and protein contents were also quantified. Operating at a extraction temperature of 200 ºC, a product with the following specification was obtained: 26.96 mg gallic acid/g extract, 28.62 mg Trolox/g extract, and 0.68 mg ascorbic acid/g extract for total phenolic content, TEAC, and FRAP, respectively. However, the highest DPPH antiradical capacity was obtained at 160 ºC with 32.85 mg/g extract. The highest concentrations of protein and free sulfates were 0.11 mg BSA/g extract and 0.87 mg/g extract, respectively. The main fatty acids found were saturated fatty acids such as palmitic acid with 81% and stearic acid with 13%, and in lower concentrations, unsaturated fatty acids with 6%. The residual solid obtained is composed of minerals such as Mg, K, Ca, and Na in high concentrations.

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Claudin 18.2-specific monoclonal antibody for cancer immunotherapy

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Gastric and pancreatic cancers rank as the 3rd and 7th leading causes of cancer-related deaths worldwide, and the 5th and 12th most common cancers, respectively1,2. Both are associated with poor prognosis, largely due to late diagnosis and limited efficacy of current treatment options. Therefore, it is essential to explore alternative therapies that are more effective and can improve outcomes for patients with these malignancies.

Claudin 18.2 is a transmembrane protein that is part of the tight junctions in the gastric epithelium. Its epitopes are mostly inaccessible in healthy tissues but are overexpressed in certain cancers, including gastric and pancreatic tumors. This restricted expression pattern makes it a promising target for immunotherapy3.

In this context, we immunized mice with virus like particles (VLP) expressing human Claudin 18.2, and developed specific mouse monoclonal antibodies targeting this protein. One monoclonal antibody, CL6, was selected based on its ability to recognize VLP-Claudin 18.2 by ELISA, while not cross-reacting with Claudin 18.1, a homologous protein expressed in lungs that differs in extracellular loop. CL6 specifically binds to an extracellular region of Claudin 18.2, as confirmed by flow cytometry in several human pancreatic and gastric cell lines and Claudin 18.2 transfected cells, but not in cell lines derived from other tissues or in non-transfected cells.

This antibody can be used directly as immunotherapy to selectively target tumor cells overexpressing Claudin 18.2, such as gastric and pancreatic cancers. Moreover, it could serve as the basis for a chimeric antigen receptor (CAR) T cell therapy, wherein T lymphocytes are genetically modified to express a CAR specific for Claudin 18.2. Upon viral transduction with the CAR-encoding sequence, these T cells could acquire the ability to recognize and eliminate tumor cells expressing Claudin 18.2 on their surface.

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Stereocontrolled total synthesis of lipofuscin pigments S-monofuran-A2E, L-monofuran-A2E and bisfuran-A2E

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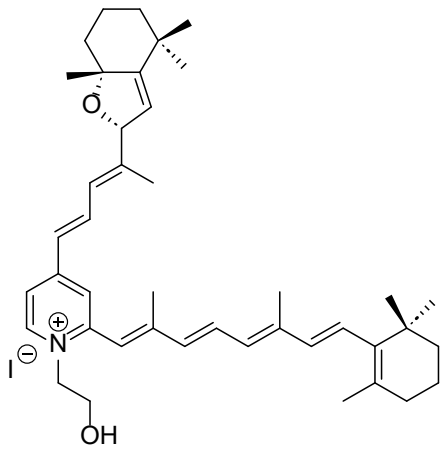
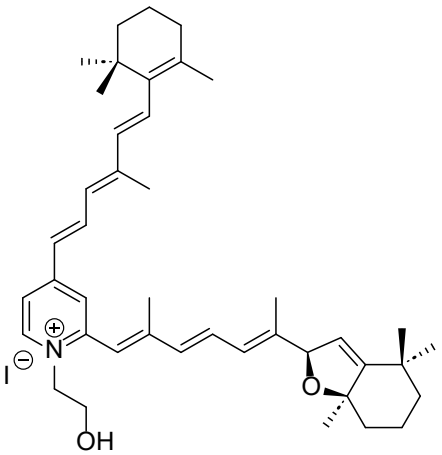
A complex mixture of inflammatory lipid-containing granules of fluorophores called lipofuscin, accumulates in the retinal pigment epithelium cells of humans, particularly those with age and certain eye-related diseases, which causes incurable blinding retinal diseases and genetic retinal disorders. Age-related macular degeneration (ARMD) is one of the degenerative diseases that affect the central area of the retina, specifically the macula, which irreversibly affects the central vision due to the deposits of lipofuscin. Not surprisingly, the World Health Organization (WHO) considers ARMD as one of the main causes of blindness in the world, and it is expected to increase due to the aging of the population, leading to stimulate efforts from the scientific community to develop treatments.[1,2]

Among the isolated and identified retinal-derived pigments, the most abundant in the macula is the bisretinoid pyridinium salt A2E.[3] Other oxidized derivatives have also been identified, among them the S-monofuran-A2E **1**, L-monofuran-A2E **2** and bisfuran-A2E **3**, but the relative and absolute configurations of the chiral centers have not been determined.[4] Although this family of compounds might also be involved in macular degeneration, the mechanism by which its accumulation in the retina leads to the disease is unknown, which retards the development of appropriate therapies to combat this pathology.

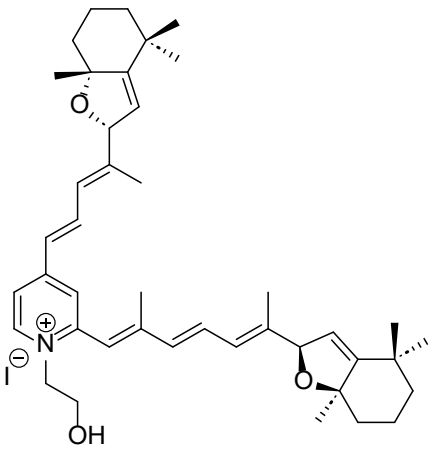
We will report on our progress on the stereocontrolled total synthesis of this pigments through a sequence of selective condensation/rearrangement/alkylation reactions. In addition to the control of the double bond geometries, the construction of the two or four chiral centers will rely on Sharpless asymmetric epoxidation and rearrangement of the epoxide to the furanoxide under controlled reaction conditions.



S-monofuran-A2E **1**



L-monofuran-A2E **2**



bisfuran-A2E **3**

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Discrimination of cancer patterns powered by surface-enhanced Raman scattering spectroscopy and classification algorithms

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Surface-enhanced Raman scattering (SERS) spectroscopy offers highly sensitive analyte detection, down to single-molecule levels [1], and exceeds traditional fluorescence techniques in multiplexing capabilities [2]. While promising in cancer detection at proof-of-concept stages, SERS lacks clinical application due to reproducibility and stability issues. Computational analysis can enhance SERS by identifying subtle spectral differences, particularly useful for complex samples like those from liquid biopsies. This study presents a hybrid plasmonic nanosensor using gold nanostars (GNS) in a porous hydrogel to distinguish SERS signals of prostate cancer (PCa) and healthy samples. GNS sterilization was optimized for safe biological use, and the sensor's properties were thoroughly characterized. Raman reporters and biological surrogates were tested to assess SERS efficiency. Machine learning (ML) was employed to classify samples based on SERS profiles, comparing signals from standalone samples and those in contact with the sensor. Results showed improved diagnostic performance using the sensor, with up to 94% AUC for cell supernatants, compared to 61% without the sensor, and 92% AUC in mice plasma. This indicates strong potential for a more sensitive, less invasive cancer diagnostic method.

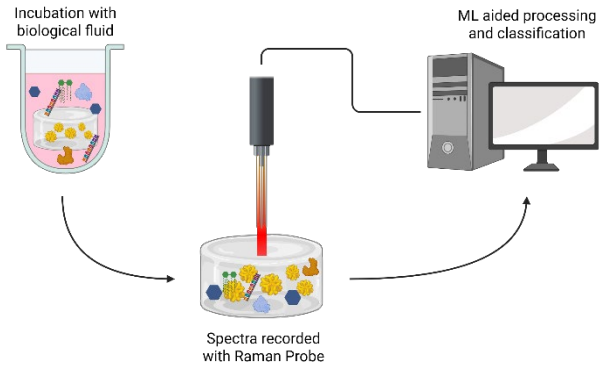

Figure 1. Descriptive protocol of the use of the nanosensor in biologically relevant samples.

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Wireless modulation of stem cell mechanotransduction signaling using PIEZO-imprinted nanoswitches

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PIEZO mechanoreceptors are essential for functions such as touch sensation, proprioception, and musculoskeletal biomechanics. Precisely targeting these receptors could unlock new strategies for tissue regeneration and disease treatment. However, current limitations in precision tools hinder a deeper understanding of PIEZO gating mechanisms and downstream signaling.[1] This study introduces "nanoswitches" – integrating molecularly imprinted polymers with magnetic nanomaterials – to enable remote, site-specific modulation of PIEZO subdomains.

Using computational epitope screening, specific regions of PIEZO proteins were selected to create PIEZO-Imprinted NanoSwitches (PINS). These were functionalized with superparamagnetic iron oxide nanoparticles to allow magnetic responsiveness. Various cell types were cultured with PINS and exposed to cyclic magnetomechanical stimulation. PIEZO activation was assessed by tracking YAP nuclear translocation and measuring PIEZO-regulated gene expression through qPCR. Transcriptomic profiling of PINS-stimulated mesenchymal stem cells further illuminated their broader biological responses.

PINS demonstrated sub-nanomolar binding affinities in surface plasmon resonance assays, while effectively targeting PIEZO *in vitro* similarly to antibodies, when observed by fluorescence microscopy. Magnetic stimulation enhanced nuclear localization of YAP and upregulated PIEZO-associated gene expression, validating the system's ability to deliver mechanical cues specifically to PIEZO channels. Notably, selective activation of different PIEZO subdomains triggered distinct gene expression profiles, including pathways associated with musculoskeletal lineage commitment – suggesting region-specific functions within the PIEZO architecture.

These findings reinforce the evolving perspective that PIEZO receptors do not simply operate through binary gating, but instead engage in context- and domain-specific signaling. The prolonged activation enabled by PINS also more closely mimics physiological mechanical stimulation than conventional short-term experimental methods. Altogether, this nanoswitch platform offers a powerful and adaptable approach to investigate PIEZO-driven mechanobiology and holds significant potential for advancing regenerative medicine, especially in mechanically dynamic tissues such as cartilage, tendon, heart, and vasculature.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon Europe research and innovation programme (Grant agreement No. 101171765 – FORTIFy); from the Portuguese Foundation for Science and Technology (FCT) (project DOI: 10.54499/2022.05526.PTDC); from Xunta de Galicia (grant ED481B2019/025); and from the Swiss National Science Foundation (Sinergia Grant Number 10000132).

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Bioelectronic implant for spinal cord stimulation and drug delivery in rats

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Spinal cord injury (SCI) remains a critical medical challenge. Although survival rates have significantly improved in recent decades, regeneration and functional recovery of damaged nerve cells are still limited. Epidural spinal cord stimulation is a promising strategy to restore motor function after SCI, but precise control over stimulation sites and parameters remains a major challenge. To develop and evaluate new therapeutic strategies, we propose a multifunctional bioelectronic implant for use in a rat SCI model. Our approach uniquely combines epidural electrical stimulation with localized drug delivery in a single device, targeting SCI in its early stages to harness neuroplasticity and promote neural regeneration. The core of the implant is a flexible, miniature stimulation probe based on commercially manufactured flexible printed circuit board (flex-PCB) and polydimethylsiloxane (PDMS). To enable drug delivery, we integrated a microfluidic channel made of PDMS, microfabricated using a silicon master mould with well-defined dimensions. The microchannel was bonded to the probe using oxygen plasma activation followed by a silane-based chemical treatment, ensuring a strong covalent bond and robust integration with the stimulation probe. When selecting materials for the stimulation electrodes, key requirements include excellent electrical conductivity, biocompatibility, and long-term stability to ensure both the safety and efficacy of the device. To meet these demands while avoiding costly and time-intensive cleanroom processes, we converted polyimide into conductive porous carbon through direct laser scribing in ambient conditions using a commercial CO_2 laser system. [1] This process produces a carbonized interface that integrates with the gold, enhancing electrode performance for stimulation applications and improving surface characteristics for electrical interfacing. This fabrication process is cost-effective, scalable, and does not depend on highly specialized instrumentation. As such, it holds potential for a broad range of implantable medical devices and wearable technologies beyond SCI. We believe this platform will support biomedical research and ultimately contribute to the development of improved therapies for spinal cord injury patients.

***Figure 1**. Bioelectronic implant bonded to a microfluidic channel, enabling combined electrical stimulation and localized drug delivery in rats.*

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Metallo-Supramolecular Fibers from Chiral OPEs Derivatives: Ag-Cation Induced Self-Assembly

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A novel methodology for the preparation of a new type of metallo-supramolecular fibers is here reported. These fibers are generated by combining a phenylacetylene monomer together with a silver salt. The resulting complex undergoes self-assembly in situ, and by adjusting the ratios of the monomer/silver (I) ratio is possible to control the fiber morphology, ranging from individual fibers to three-dimensional networks1,2.

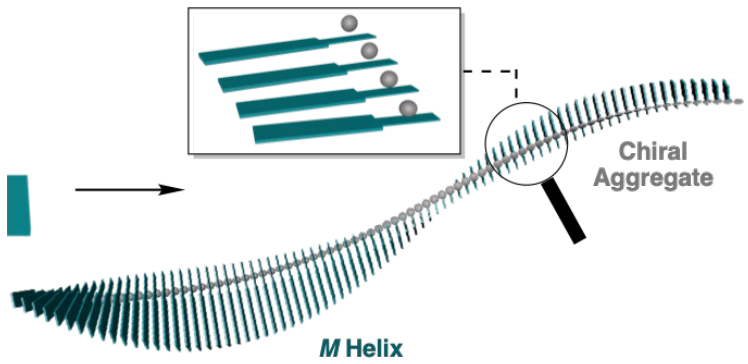

Figure 1. Ag-cation aggregation of a phenylacetylene monomer.

Financial support from AEI (PID2022-136848NB-I00), Xunta de Galicia (ED431C 2022/21, Centro Singular de Investigación de Galicia acreditación 2023–2027, ED431G 2023/03, ED431G 2023/06) and the European Regional Development Fund (ERDF) are gratefully acknowledged. H. L. G. thanks AEI for a FPI contract.

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CO_2 detection with porphyrins in surface-enhanced Raman scattering (SERS) through Artificial Intelligence methods

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Monitoring carbon dioxide (CO_2) in aquatic environments has become increasingly critical due to its environmental and industrial relevance [1]. Surface-Enhanced Raman Scattering (SERS) has emerged as a promising analytical technique for this application, offering high sensitivity and selectivity, along with practical advantages such as low cost and ease of implementation. However, due to of its low Raman cross-section and lack of affinity for plasmonic noble metal nanostructures, direct SERS detection of CO_2 remains challenging. To address this, SERS substrates combining porphyrins, as selective chemoreceptors, with gold nanostars that serve as optical enhancers were developed. Thus, the identification of dissolved CO_2 was performed by analysing the spectral variation of SERS fingerprints of porphyrins when the interaction between CO_2 and porphyrins occurs [2].

Recognizing the increasing role of data-driven methodologies in analytical chemistry, we further integrated a machine learning (ML) pipeline to automate and improve CO_2 detection. Our approach includes preprocessing of portable, in-house SERS data, data augmentation to boost model robustness, and both supervised and unsupervised ML algorithms for feature extraction and classification. Through feature engineering and hyperparameter optimization, we achieved a classification accuracy of 96.3% using an Extra Trees model [3]. This interdisciplinary framework bridging nanomaterials, spectroscopy, and AI, presents a robust strategy for enhanced CO_2 sensing in water systems, contributing to advances in environmental monitoring technologies.

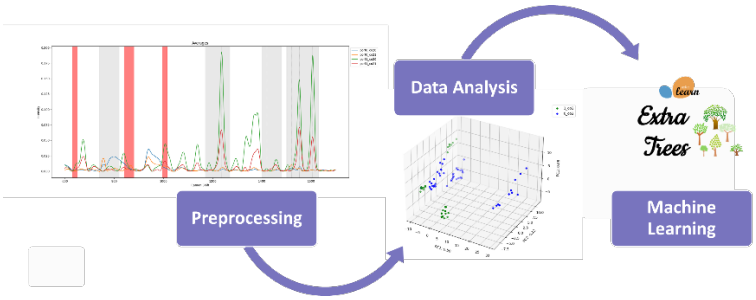

Figure 6. Pipeline description towards AI-driven classification of CO_2 with porphyrins in SERS.

Acknowledgement of Funding

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Computational and experimental exploration of plasmonic catalysts for water splitting and ammonia synthesis

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The global carbon dioxide crisis forces us to look for sustainable, renewable, clean, and environmentally friendly energy. The sun not only provides light, warmth and sustains life on Earth, but it is also an abundant and renewable source of energy that we can use to drive diverse facets of our civilization. Solar-to-chemical energy conversion can be a key technology in driving a renewable future, if we achieve significant energy conversion efficiencies. Using plasmonic materials can help improve it, as they interact strongly with light and have become a salient class of light harvesters. A plasmon is a resonant, collective oscillation of free electrons at the surface of a metal, typically excited by light. They can be used in photocatalytic approaches to water splitting and ammonia synthesis. Water splitting is interesting from the point of view of advancing the production of green hydrogen, but is also the relevant background for other photocatalytic reactions pursued in aqueous solution. Ammonia is a strategic material widely used as a reagent in the synthesis and production of various agricultural products, and it would be enormously beneficial to produce it at low temperatures, atmospheric pressure and in, precisely, aqueous solution.

I will present my current progress on modelling water splitting on gold (a common plasmonic material) surfaces using Density Functional Theory (DFT) techniques, and in testing catalytic synthesis of ammonia with photocatalysts consisting in gold nanostructures encapsulated within a metal-organic framework (MOF).

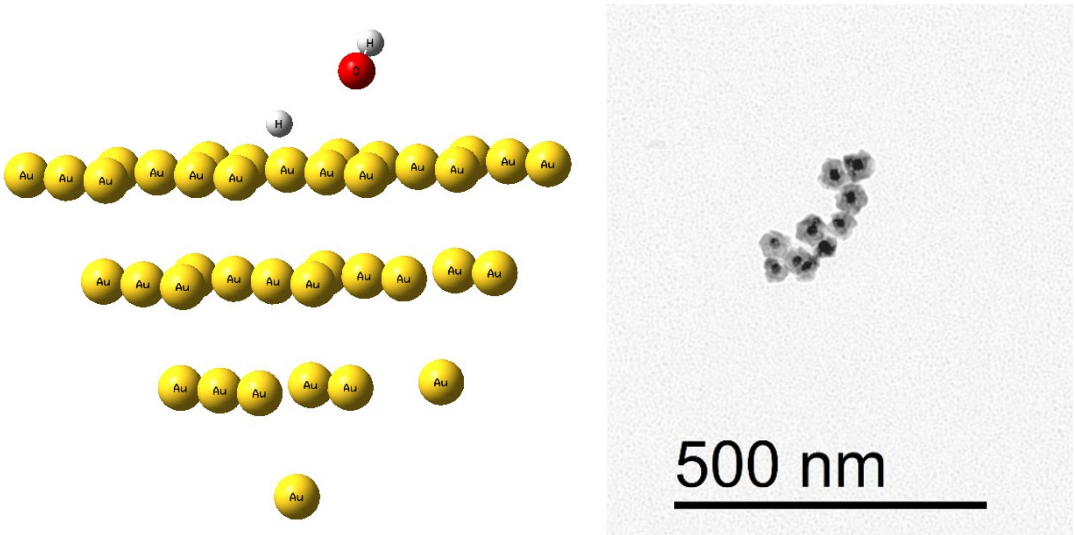

Figure 1. (Right) Au@Cu2O for encapsulation in MOF. (left) water splitting on the surface of a gold pyramid structure.

Evaluating Nanoparticle Binding and Release on Functionalized Graphene under Hydrodynamic Flow

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Understanding the binding strength between nanoparticles (NPs) and functionalized surfaces is crucial for the development of robust nanomaterial-based sensing platforms. In this study, we systematically examined the attachment and detachment behaviour of fluorescent NPs on graphene surfaces non-covalently modified with pyrene derivatives. To evaluate the hydrodynamic forces involved, finite element method (FEM) simulations in COMSOL Multiphysics were used to model laminar flow conditions and quantify the resulting drag and lift forces acting on 100 nm particles. Two NP materials were tested: polystyrene (PS)-based and silica (SiO_2)-based; with two functionalization strategies: (i) electrostatic interactions between carboxylated NPs and NH_2-pyrene-functionalized graphene, and (ii) high-affinity biotin–Neutravidin binding via pyrene–biotin modification. Interaction dynamics were monitored in real time using a custom-designed microfluidic flow cell coupled with confocal fluorescence microscopy under increasing shear flow. While PS-based NPs showed strong non-specific adhesion, likely due to π-π stacking with the graphene surface, SiO_2-based NPs enabled controlled binding and detachment, allowing accurate assessment of specific interaction forces. These results underscore the importance of nanoparticle composition, surface chemistry, and hydrodynamic conditions in tuning NP-surface interactions, and provide a versatile platform for studying molecular detachment forces relevant to biosensing applications.

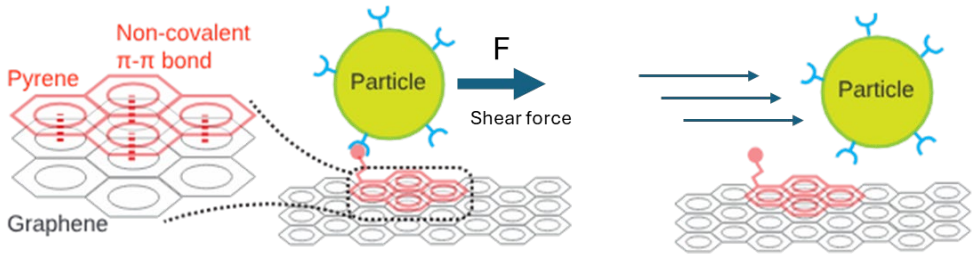

Figure 1. General scheme of Nanoparticle release

Acknowledgements: 101130125 — FLUFET — HORIZON-EIC-2023-PATHFINDEROPEN-01

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Investigation of Stepwise Photocatalytic Degradation of Rhodamine B: Optical Modelling through First Principles

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Dyes are common materials used for measuring the performance of different photocatalysts, as their degradation can be easily tracked with optical measurements. Different types of dyes are used such as methylene blue, methyl orange and Rhodamine B (RhB) [1]. Among these dyes, RhB is often used in photocatalytic degradation study because of its stability, resilience against light-induced fading and vivid coloration [2]. Photocatalyst such as TiO2 can absorb photons that promote the formation of electron-hole pairs. These charge carriers can drive redox reactions with oxygen and water to form reactive oxygen species (ROS), including hydroxyl radicals (•OH) and superoxide radicals (•O2-). These ROS are extremely reactive and target organic substances such as RhB, breaking down their chemical structure into smaller molecules. We discuss experimental [3] and ab initio (DFT + GW-BSE) characterization of the photocatalytic degradation of RhB. These findings contribute to a deeper understanding of the photocatalytic degradation of organic dyes, which is crucial for developing more effective photocatalysts for environmental cleanup and waste water treatment applications.

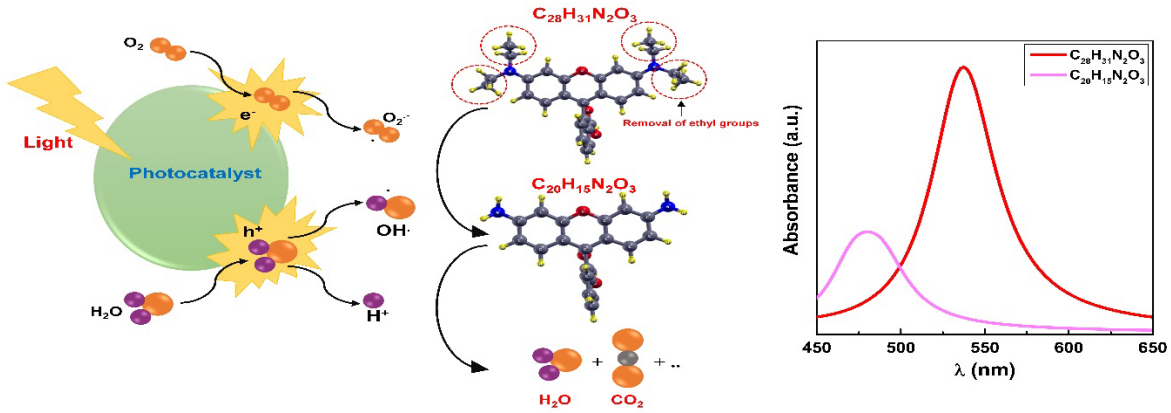

Figure. *(Left) RhB before and after a multi-step degradation process, highlighting the groups lost along the process. (Right) Computational results for the optical absorption of both molecules.*

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Single cell-derived spheroids SERS-based metabolomic profiling

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CTCs are highly heterogeneous, displaying distinct characteristics in terms of their genomic, proteomic and metabolomic features, which result in varying levels of metastatic capability[1]. In our work, we focus on the metabolome of CTCs and how it can be related with their metastatic potential[2]. For metabolomics analysis, we aim to use surface enhanced Raman spectroscopy (SERS) - an untargeted, ultrasensitive technique that employs plasmonic nanoparticles, such as gold nanostars (GNSs), to enhance Raman signal from metabolites secreted cells[3]. To enable a more comprehensive single-cell profiling and overcome limitations associated with direct single-cell analysis, we developed a model to expand single cells into single cell-derived spheroids, cultured within an adipose tissue-derived extracellular matrix (adECM). To this end, we used the non-cancerous MCF-10A and breast cancer (BC) MCF-7 cell lines, respectively. MCF-7 and MCF-10A cells successfully grew into single cell-derived spheroids with an average size of 187.2±195.2 µm and 95.4±53.8 µm, respectively. Additionally, GNSs were introduced in the hydrogel matrix at different concentrations (0.5, 2, and 4 mM) to test their biocompatibility - MCF-7 spheroids showed no significant difference in the presence of GNSs concentrations up to 2 mM in terms of diameter, metabolic activity and DNA amount; at 4 mM GNSs , however, spheroids were significantly smaller, less metabolically active , with significantly reduced total DNA content. Similar results were obtained for the MCF-10A cell line in the presence of GNSs. When expanding this method for different breast cancer subtypes, distinct behaviours were observed: BT-474 cells grew small spheroids with and without the presence of GNSs, SKBR-3 spheroids were significantly affected by GNSs presence and MDA-MB-231 cells did not grow into spheroids, displaying a more invasive-like growth.

In conclusion, GNSs concentrations up to 2 mM did not significantly affect MCF-7 and MCF-10A spheroid growth, validating our method for SERS measurements. Besides, the different behaviours of the other BC subtypes highlight BC phenotypic heterogeneity, possibly predicting the different behaviours we will find when working with primary CTCs.

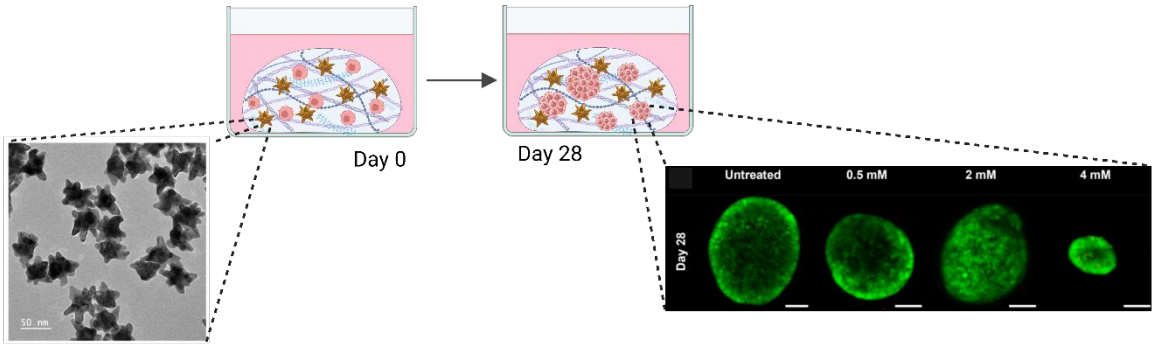

Figure 1. Single MCF-7 cells are seeded within the hydrogel at day 0 in the presence of different concentrations of GNSs (left image). At day 28, a Live/Dead assay is performed to access spheroid growth kinetics (magnification: 20x; scale bar: 100 µm).

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Chiroptical stimuli-responsive behavior of covalent polyacetylene chiral polymers

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Helical polymers are promising materials with wide-ranging applications in asymmetric catalysis, chiral recognition, sensing, optoelectronic devices, and biological systems. [1] Among them, poly(phenylacetylene)s (PPAs) are dynamic helical polymers capable of tuning their P/M helical sense and compression/elongation in response to external stimuli such as pH, temperature, solvent polarity, chiral additives, and metal ions. [1,2] The helical backbone is not rigid but dynamic, allowing external stimuli to influence not only the induced helical sense of the polymer but also the stretching or compression of the helical scaffold. Thus, chiral amplification toward a specific helical sense or helical inversion between P and M helices can be accompanied by the elongation or compression of the helical structure. The functional groups present in the pendant will be oriented in a specific position towards the helix. [2,3] The spatial arrangement of pendant functional groups along the polymer backbone plays a critical role in controlling these transitions, enabling helical inversion, screw-sense amplification, and helical structural modulation through supramolecular interactions and cooperative effects. [2,3] While polyacetylene containing aromatic phenyl groups as PPA's have been extensively explored, their aliphatic-unsaturated counterparts remain relatively underexplored. To address this gap, we introduce a novel class of water-soluble unsaturated chiral polyacetylene polymers in both homo and copolymer forms. Their chiroptical properties are demonstrated to exhibit reversible switching behavior and compression-elongation of helical screws in response to external stimuli, including the formation of chiral complexes. These findings contribute to the fundamental understanding of dynamic helical polymers and pave the way for their potential applications in smart materials and responsive molecular systems.

Keywords: Chiral, dynamic helical polymer, polyacetylene, and stimuli-response.
Funding:

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Comprehensive characterization of graphene-based ion-to-electron transducers for development of all-solid-state lithium-selective electrodes

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All-solid-state ion-selective electrodes (ISEs) have been improved by the incorporation of nanostructured materials that facilitate chemical-to-electrical signal conversion, increase potential stability, and prevent water layer formation [1]. Among them, carbon nanomaterials, known for their good electrical conductivity, high hydrophobicity, chemical stability, low-cost, and ease of functionalization, are considered ideal solid-state transducing layers. Representative examples include carbon nanotubes, fullerenes, carbon nanofibers, and graphene (GPH). Nevertheless, the high versatility of GPH attributed to different synthesis methods and subsequent impact on the electrical properties, drove the attention of researchers to understand its potential in the development of robust potentiometric sensors [2].

Accordingly, this work aims to extensively characterize, in one parallel study, the physico-chemical properties of different GPH-based transducers in custom-made and commercially available electrodes to develop all-solid-state lithium-selective electrodes (Li-ISEs). Custom-made electrodes were obtained by i) screen-printing carbon-GPH ink onto a polyethylene terephthalate substrate (SPCGE/PET) and ii) direct laser irradiation of polyimide substrates to produce Laser-Induced Graphene (LIG/PI) [3]. On the other hand, commercially available GPH-modified screen-printed electrodes on ceramic substrates (SPGE/CR) were acquired from Metrohm Dropsens® (DRP-110GPH).

An extensive characterization of the bare electrodes was carried out in terms of morphology, structure, composition, hydrophobicity, and electrochemical performance. The corresponding Li-ISEs were prepared by surface modification with a lithium sensing membrane and the potentiometric performance was evaluated in steady-state configuration against an external Ag/AgCl reference electrode. A Nernstian response was observed for the SPCGE/PET and SPGE/CR-based Li-ISEs in the broad linear response range from $10^{-5.5}$ to 10^{-1} M, with a slope of 53.8 ± 1.9 and 61.9 ± 1.2 mV dec^{-1} and a limit of detection of 1.2 x 10^{-6} and 3.2 x 10^{-6} M, respectively. The water-layer test revealed a corresponding long-term potential drift of 0.1 and 0.5 mV h^{-1}. In comparison, the LIG/PI-based Li-ISEs demonstrated a lower sensitivity (34.9 ± 2.5 mV dec^{-1}), a limit of detection of 5.5 x 10^{-6} M, and a potential drift of 3.7 mV h^{-1}, which can be attributed to its high surface porosity.

This study highlights the impact of the properties of GPH on the potentiometric performance of the corresponding all-solid-state ISEs. The future work comprises the optimization of LIG conditions to develop robust and stable sensors.

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Periodic Nanostructured Arrays of Gold Nanoparticles as SERS substrates

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Surface-enhanced Raman scattering (SERS) spectroscopy stands as an analytical tool with great potential, offering exceptional sensitivity for the detection of various analytes. SERS detection is based on the amplification of Raman signals, primarily due to the strong electromagnetic fields present on plasmonic surfaces.[1] However, the implementation of SERS in industrial applications has been limited by the challenges associated with obtaining SERS substrates that are efficient, robust, homogeneous, and reproducible. To address these limitations, SERS substrates based on periodic arrays of gold nanoparticles have emerged as a promising solution. These nanostructured arrays exhibit narrow and intense optical properties, enabling strong plasmonic coupling and field enhancement.

We report a large-scale synthesis of gold nanoparticles using a seeded-growth method, providing precise control over nanoparticle size and morphology. These nanoparticles are arranged into one-dimensional periodic nanostructures using the stamping methodology (Figure 1A).[2] The resulting nanostructures are evaluated for their potential as surface-enhanced SERS substrates using a model molecule.

Our results demonstrate that we successfully achieved the desired periodic arrangement of nanoparticles (Figure 1B), allowing us to effectively tune their optical properties (Figure 1C) through the coupling of plasmonic modes.[2,3] This tunability enables the development of customized, high-performance SERS substrates tailored for specific applications. Additionally, tests showed that the substrates provided consistent signal enhancement across different areas and during repeated measurements, confirming their homogeneity and reproducibility.

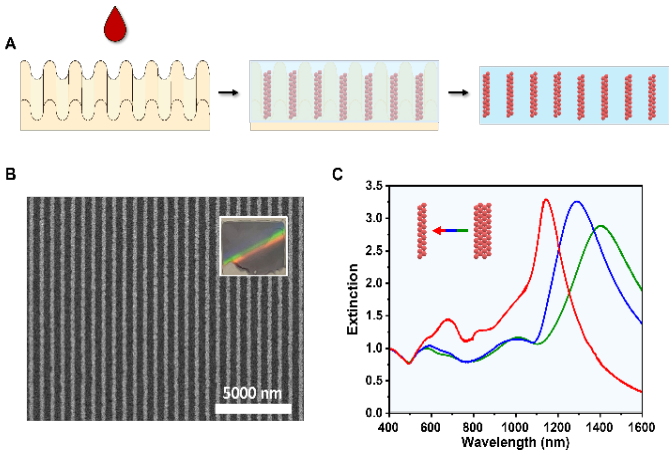

Figure 1. Schematic diagram of the SERS substrates fabrication process employing the stamping methodology (A). SEM image (B) and extinction spectra of the 1D (C).

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Expansion of π-System of Covalent Organic Frameworks via Cyclodehydrogenation

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π-Conjugated materials stand out due to their remarkable optical and electronic properties. In this context, covalent organic frameworks (COFs) offer an ideal platform for the precise organization of structural units within a π-conjugated crystalline network. This architecture enables the modulation of properties through the selection of functional groups, either prior to COF formation or via post-synthetic modifications.[1] An effective strategy to extend π-conjugation is cyclodehydrogenation, in which diketone units in the COF react with aromatic *o*-diamines to yield azaacene COFs with enhanced photophysical properties.[2] In previous work, it was demonstrated that this transformation can also be achieved via a multicomponent reaction, allowing access to highly crystalline boronic ester COFs featuring pyrene-fused azaacene motifs.[3]

Herein, we report on the cyclodehydrogenation of an imine-linked COF incorporating phenanthrenequinone units. Initially, model systems were studied to gain insight into the intermolecular interactions between the azaacenes. Subsequent optimization of the synthetic conditions through systematic screening enabled the preparation of a crystalline phenanthrenequinone-based imine COF. Using a multicomponent approach, an *o*-diamine was incorporated into the reaction mixture to form π-conjugated COFs. With crystalline aza-phenanthrene COFs in hand, structural and optical characterizations were carried out to evaluate the influence of π-extension on their properties.

Acknowledgement of Funding
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Bi-metallic organic frameworks encapsulated in nano fiber for antimicrobial food packaging applications

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The development of advanced food packaging materials is essential for enhancing food safety, extending shelf life, and reducing environmental impact. In this study, a novel approach involving the encapsulation of bimetallic frameworks (BMFs) in electrospun nanofibers is proposed for smart food packaging applications (Figure 1). The objective is to improve the packaging membranes by increasing their protection against microorganisms and their mechanical strength.[1]

Bimetallic frameworks (BMFs), known for their superior antimicrobial activity, catalytic properties, antioxidant efficacy, and stability were synthesized via one-step solvothermal method at room temperature using a combination of metal ions such as nickel, cobalt and zinc. These BMFs were then incorporated into biodegradable, biocompatible and antibacterial polymer matrices of Chitosan and Poly ethylene oxide (PEO) via electrospinning, that consist in a simple, versatile and cost-effective fiber fabrication technique based on electrostatic interactions. An optimization of the key parameters affecting the electrospining process, such as voltage, concentration, flow rate and the distance between the needle and the collector, was performed to obtain membranes with a homogenous distribution of the BMFs as well as with the desired mechanical properties.

TEM, SEM, DLS, XRD and FTIR characterization techniques were used to confirm the successful formation of the structures. Agar disc-diffusion techniques will be developed to test antimicrobial activity.

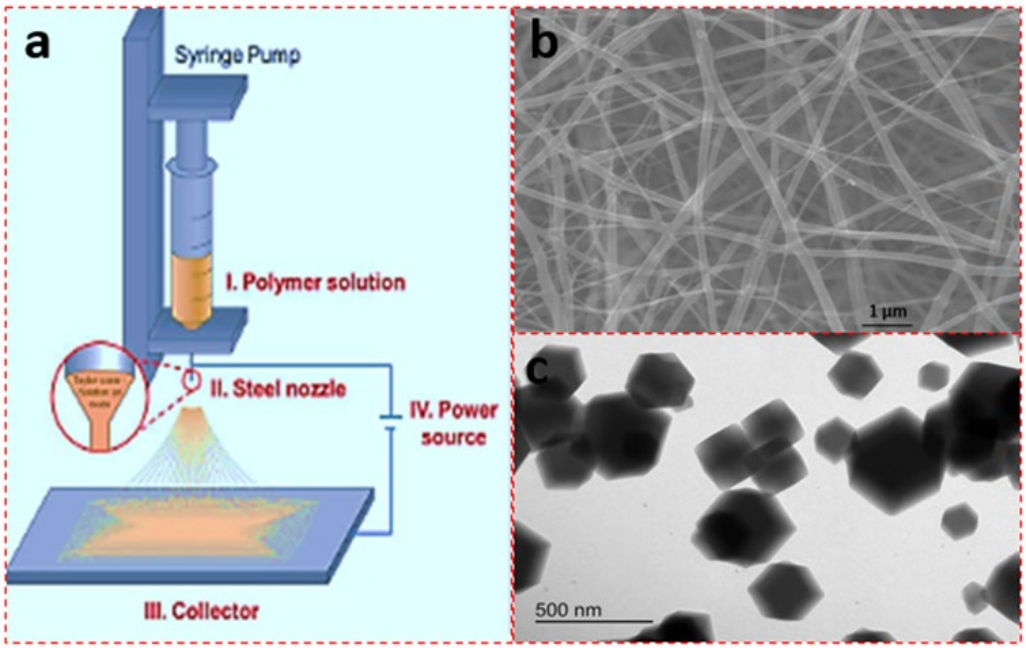

Figure 1. (A) Electrospinning technique. (B) SEM of a PEO/Chitosan membrane and (c) TEM of BMFs.

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Single administration of Transforming Growth Factor Beta 3 via poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanoparticles for cartilage repair

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Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is a biodegradable, biocompatible biopolymer. This study assessed PHBV nanoparticles (NPs) as a delivery system for transforming growth factor β3 (TGF-β3), aiming to promote mesenchymal stem cell (MSC) chondrogenesis in cartilage repair. Key evaluations included cytotoxicity, release kinetics, and cellular responses in two-dimensional (2D) and three-dimensional (3D) models, as well as an ex vivo model.

Cytotoxicity assays following ISO 10993-5:2009 and ISO 10993-12:2021, using human keratinocyte cells (HaCaT) and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, showed that materials purified with chloroform and 86% ethanol were non-cytotoxic, with high cell viability: 85.69 ± 14.17% (single precipitation) and 87.71 ± 3.58% (double). Enzyme-Linked Immunosorbent Assay (ELISA) showed sustained TGF-β3 release from NPs over 29 days, with a peak on day 4 (235 pg/mL) and a daily average of 143.21 ± 41.07 pg/mL. In 2D cultures, TGF-β3-loaded NPs (added at the beginning of the experiment) significantly enhanced COL II expression (3.07 ± 0.19 a.u.) compared to free TGF-β3 (added twice a week) (1.38 ± 0.04 a.u., $p < 0.0001$) and reduced COL I expression (0.77 ± 0.04 a.u.) versus the free TGF-β3 group (1.38 ± 0.12 a.u., $p < 0.0026$), indicating a more chondrogenic and less fibrotic profile. In 3D micromass cultures, the free TGF-β3 group showed the highest collagen (78.23%) and proteoglycan (62.98%) deposition. The TGF-β3 NP group showed lower matrix formation (11.34 ± 3.44% and 13.50 ± 4.26%), likely due to reduced cell–matrix interaction, but still outperformed the empty NP group (7.74% and 7.01%). The pericellular lacunae characteristics of cartilage tissue was still observed in the TGF-β3 NP group, as in the free TGF-β3 condition. In the ex vivo repair model, the total ICRS II repair score based on six parameters (tissue morphology, matrix staining, cell morphology, chondrocyte clustering, surface architecture, and baseline integration) was highest for TGF-β3 NPs (64%) compared to free TGF-β3 (51%) and empty NPs (46%).

In conclusion, PHBV NPs enabled sustained TGF-β3 delivery and supported MSC chondrogenesis. A single dose achieved outcomes comparable or superior to repeated free TGF-β3, suggesting a simpler, safer strategy for cartilage repair.

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Structural and Magnetic Modulation of Akaganeite Nanorods via Controlled Chloride Doping

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Akaganeite (β-FeOOH) is a tunnel-structured iron oxyhydroxide that has attracted increasing interest across various scientific fields due to its physicochemical properties and its role in corrosion processes and saline environments. At the nanoscale, it exhibits a high surface-to-volume ratio and enhanced reactivity, making it relevant for environmental, catalytic, and energy storage applications. Additionally, its antiferromagnetic behavior at low temperatures positions it as a promising material in magnetic nanomaterial studies. [1]

This iron oxide is synthesized via a hydrothermal process using iron(III) chloride ($FeCl_3$) as a precursor, which results in chloride ions being incorporated within its tunnel structure. These chloride ions can be identified through Raman spectroscopy, as they are associated with two characteristic spectral bands at 310 and 330 cm^{-1}. A higher intensity ratio between these bands correlates with a higher chloride concentration in the nanoparticles, and vice versa. [2]

To enhance the magnetic properties of this material, acicular akaganeite nanoparticles (~600 nm in length and ~130 nm in width) were synthesized and subjected to a treatment in the presence of sodium hydroxide (NaOH) to replace chloride ions with hydroxide ions. This treatment was performed for different reaction times (30 min, 1 h, 2 h, 3 h, and 8 h) and analyzed via Raman spectroscopy, to evaluate whether the intensity ratio of the characteristic bands evolved in accordance with the chloride ion concentration, and via magnetometry. [3]

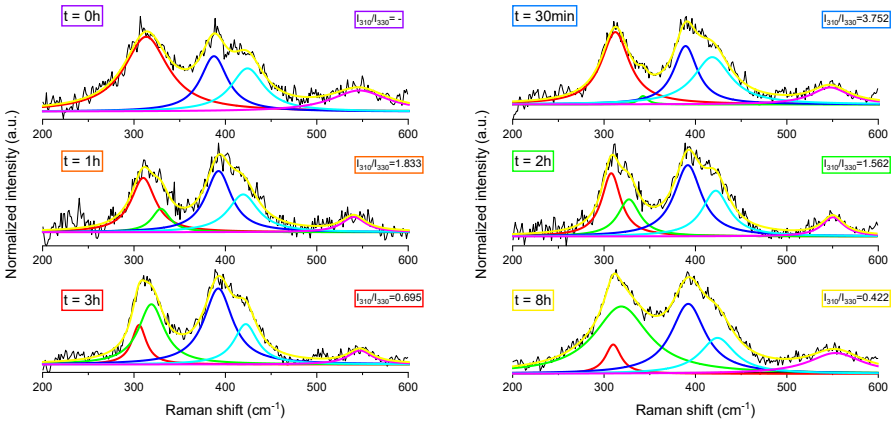

Figure 1. Raman spectra of the akageneite nanorods synthesized and subjected to a chemical treatment using NaOH at different times.

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Innovative Plasmonic Nanolipogel Platform for Multimodal Cancer Therapy

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Monotherapeutic nanodelivery systems have shown limited effectiveness in cancer treatment. In contrast, multifunctional nanosystems offer a promising strategy to overcome drug resistance and improve therapeutic outcomes. Among these, nanogels have attracted attention as drug carriers due to their biocompatibility and high drug-loading capacity [1,2]. When combined with plasmonic nanoparticles, they enable controlled and sequential drug release in response to specific stimuli [3].

In this work, we propose a multifunctional nanosystem designed to address this complex challenge. It consists of mesoporous silica-coated gold nanorods capped with a lipid layer, along with glutathione (GSH)-responsive nanogels possessing photothermal capabilities (Figure 1). The system was developed to enable the controlled and stepwise release of a commercial chemotherapeutic agent (mitoxantrone) and a glucose analogue (2-deoxy-D-glucose, 2-DG). The glucose analogue is intended to inhibit glycolysis, thereby reducing the expression of heat shock proteins and helping to overcome resistance to hyperthermia induced by near-infrared (NIR) laser irradiation. The system's stability and drug release behavior were evaluated under various stimuli, including pH, GSH concentration, and NIR irradiation.

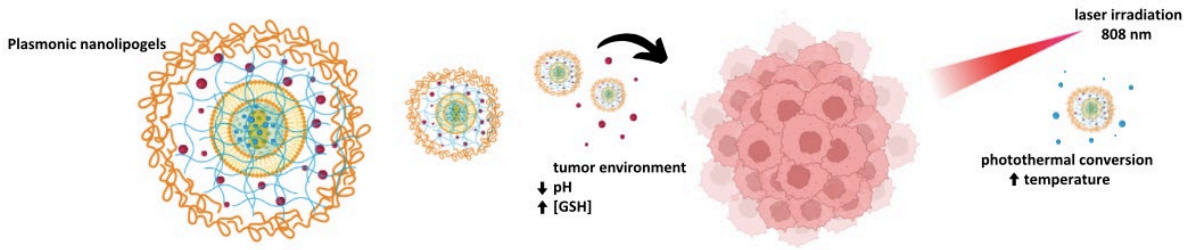

Figure 1. Scheme of the concept of the proposed nanosystem.

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Synthesis of sustainable hybrid structures based on cyanine dyes and SiO_2 particles for nanophotonics

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Sustainable photonic technologies require the development of new materials that can mimic the properties of critical traditional raw materials such as metals and semiconductors. Some organic dyes, such as cyanines, have shown an excellent potential for use in photonic materials as they can provide organic platforms with high refractive index values similar to semiconductors[1,2], or negative values of permittivity like metals[3]. These properties arise from the formation of supramolecular structures, which are assembled in a specific and highly ordered manner. In this work, we focus on supramolecular structures with a J-type conformation for being the best candidates to achieve the interesting photonic properties described above.

In this work, we use colloidal particles as templates to deposit cyanines dyes, controlling their conformation as J-aggregates at the surface of the particle. Silica (SiO_2) was selected as the template material due to its abundance, low environmental impact, and biocompatibility, making it an attractive option for sustainable photonic applications. The strategy is to combine colloidal Mie resonators based on SiO_2 particles with J-aggregates of cyanine dyes. SiO_2 particles enable the manipulation of light at the nanoscale through efficient low-loss electric and magnetic modes. To enhance and modulate their optical properties, the surfaces of these particles have been functionalized with J-aggregate cyanine dyes, integrating the absorption and emission characteristics of these organic compounds in hybrid structures.[4] We employed electrostatic self-assembly via the layer-by-layer (LbL) method to sequentially deposit cyanine J-aggregates and polyelectrolytes of opposite charge onto SiO_2 particles (Figure 1).

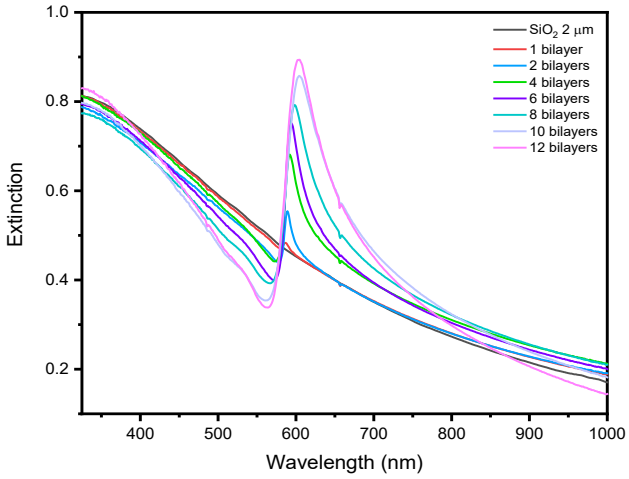

Figure 1. Extinction spectrum of 2 µm silica particles functionalized with 12 bilayers of TDBC@PDDA.

We thank the financial support from European Innovation Council and SMEs Executive Agency (grant: 101129661 – ADAPTATION).

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Development of a Raman-Based Droplet Sorter

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Determining properties of single cells has been a subject of great interest in the biomedical field for a long time, specifically in cancer research where characteristics of individual cells can affect proliferation into different types of tumours. One of the main challenges for these individual assays is efficiently separate and discriminate different types of cells. Even though fluorescent spectroscopy is the most common approach to tackle this problem, Raman Scattering spectroscopy presents great potential due to its higher sensitivity and specificity. Using Raman Spectroscopy also presents a series of challenges to address, such as lower signal intensity and lower reproducibility. Nanotechnology and microfluidics are great allies to face these challenges, being of special interest the use of nanoparticles and Surface Enhanced Raman Scattering Spectroscopy (SERS), and droplet microfluidics [1][2]. As such the main goal of my thesis is to use these multidisciplinary technologies to engineer a sorting platform capable of classifying cell-loaded droplets based on their SERS fingerprint. As a case study, we will make use of the developed platform to sort various cancer cell types and separate and isolate them according to their membrane protein expression.

This approach will be driven by designing and fabricating different microfluidic devices using 3D printing technologies for droplet formation and manipulation [3] that will be analysed using a customized Raman microscope system based on an optical fibre Raman probe [4]. The platform it's going to be user-friendly, and it is going to combine precision and efficiency in a portable modular approach being able to work in different scenarios.

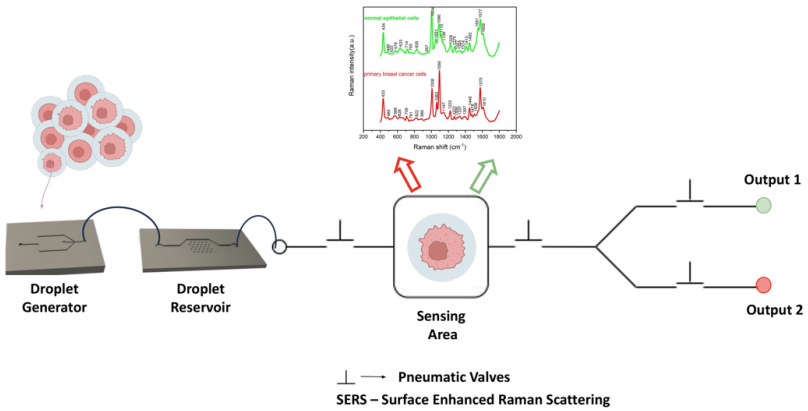

Figure 1-Integrated system of microfluidic devices for generating cell-containing droplets for SERS analysis

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Polymorphism-Controlled Structural Tuning in Ni(II)-Based MOFs: Insights from Pure and Mixed Phases

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Polymorphism refers to the phenomenon where a single chemical compound can crystallize into two or multiple structural arrangements, each exhibiting unique physical properties despite identical molecular composition.[1] In the context of porous materials, polymorphism leads to structural variations, such as differing pore geometries and surface characteristics, all derived from the same chemical constituents. This concept has gained increasing attention in the study of metal–organic frameworks (MOFs) in the past few years.[2] However, researchers have focused primarily on the pure phases of polymorphic MOFs, while intermediate-phase and mixed-phase structures have been largely overlooked due to their structural complexity and the challenges associated with controlling their synthesis. In our work, we revisit the polymorphism of Ni-BDC-DABCO MOF. In addition to the well-known pure-phase polymorphs with square (*sql*) and Kagome (*kgm*) topologies,[3] we investigate mixed-phase MOFs by various structural and gas adsorption analysis techniques, giving access to structure–property relationships. Furthermore, systematic study of the synthesis conditions allows us to unravel key parameters determining the phase composition in these mixed-phase MOFs.

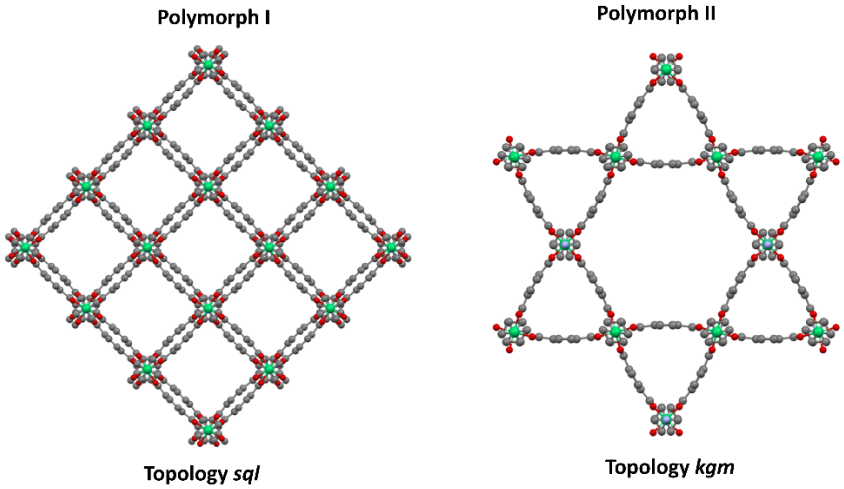

Figure 1. Two polymorphs of Ni-BDC-DABCO MOF representing sql and kgm topologies.

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MEMCAT

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Ultra-sensitive portable SERS-based system for water contaminants

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The rapid, sensitive, and selective detection of chemical compounds in complex matrices is a persistent challenge in areas such as food safety, environmental analysis, and public health [1]. Surface-Enhanced Raman Scattering (SERS) has emerged as a particularly promising technique in this context, thanks to its ability to significantly amplify Raman signals and provide detailed molecular information, even at very low concentrations. The use of nanostructured substrates allows for strong signals enhancement, while also enabling the development of compact and potentially portable systems [2,3]. These features make SERS especially attractive for contaminants detection, where rapid and reliable identification of hazardous substances is essential.

In our work, we focus on the development of portable SERS-based sensors for the detection of contaminants in different water sources. Considering the complexity of real water sample matrices, we are developing strategies to tailor SERS substrates, enabling efficient molecular recognition and subsequent analysis using portable Raman systems. To further enhance sensitivity and selectivity, we are also integrating artificial intelligence approaches to assist in distinguishing between different molecular signatures, improving both detection accuracy and robustness in real-world samples.

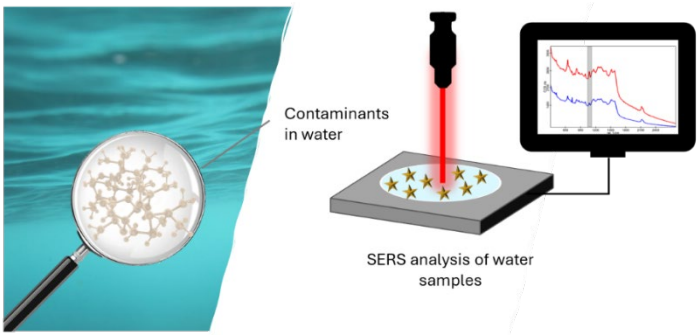

Figure 1. Schematic illustration of the sensing platform.

Acknowledgments:

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Use of IoT in Data Acquisition of a Calvet Microcalorimeter

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The Calvet microcalorimeter allows the study of physical-chemical-biological processes of various durations [1-3]. Its structure includes a thermostatic system with multiple chambers and a detector system formed by two identical thermopiles. The implementation of IoT technologies has enabled remote access to this device, improving continuous data monitoring from remote locations. Significant advances have been made in the automation of data acquisition with this system, through a remote interface connected to a microcalorimeter. To perform the measurements, a microvoltmeter connected to an ESP32 microcontroller is used, which transmits the data to a remote server using the MQTT protocol. The data is stored in a SQL database and is accessible through a web frontend developed with Angular. This frontend allows users to monitor experiments in real-time and perform detailed analyses of the recorded data. The use of these technologies not only improves the efficiency of data collection but also allows greater flexibility and accessibility for researchers.

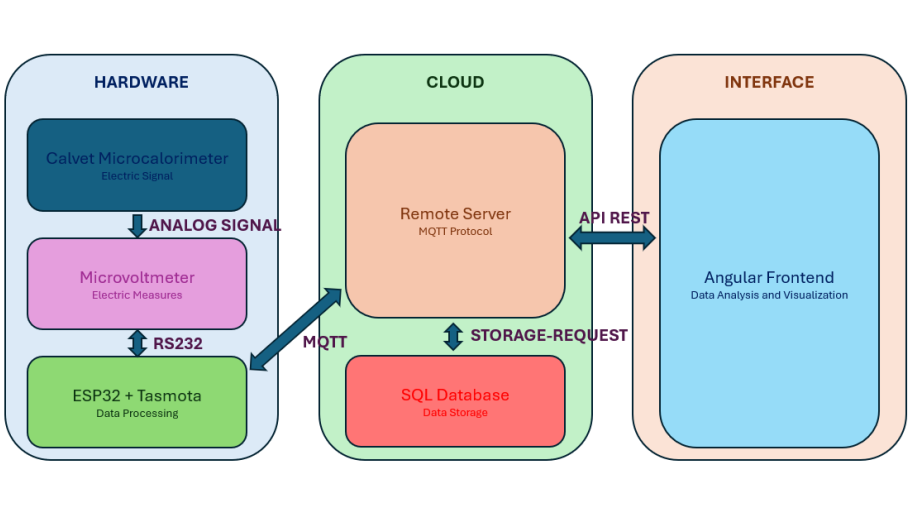

Figure 1. Architecture of the Data Acquisition System

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Deciphering the Evolution of Ni Doping on ZIF-8 via Computational Simulations: A Structure-Property Relationship

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Metal-organic frameworks (MOFs) especially zeolitic imidazolate frameworks (ZIFs), have emerged as highly promising photocatalysts, because of their unique structural features and tunable properties. ZIF-8 stands out as one of the most frequently investigated ZIF materials [1]. However, its wide bandgap limits light absorption to the ultraviolet region, and rapid charge carrier recombination adversely affects photocatalytic efficiency [2]. To overcome above challenge, it is essential to strategic doping with metals (Ni2+ substitution at Zn2+ sites) help to narrowing the bandgap and improve overall performance [3]. These modifications enhance light absorption into the visible spectrum while preserving the structural stability of the framework. To investigate and predict these effects, a Density Functional Theory (DFT) study has been conducted. In ZIF-8, each zinc (Zn) center is tetrahedrally coordinated by four 2-methylimidazolate ligands, with one nitrogen (N) atom from each ligand forming a bond with the Zn atom. However, in Ni-doped system (NiZIF-8), one Zn atom in the unit cell is substituted with a Ni atom. The structural and electronic properties of the ZIF-8 and NiZIF-8 were computed using Perdew-Burke-Ernzerhof (PBE) generalized gradient approximation (GGA) implemented in Quantum Espresso program suites [4-5]. Our findings confirm that NiZIF-8 shows a markedly reduced band gap compared to the pure ZIF-8 system. Moreover, the introduction of Ni atom not only narrows the bandgap but also reshapes the orbital distribution, enhancing metal-orbital interactions in the conduction band. The shift in orbital localization suggests possible electronic transitions, which promote charge transfer processes beneficial for light-harvesting applications.

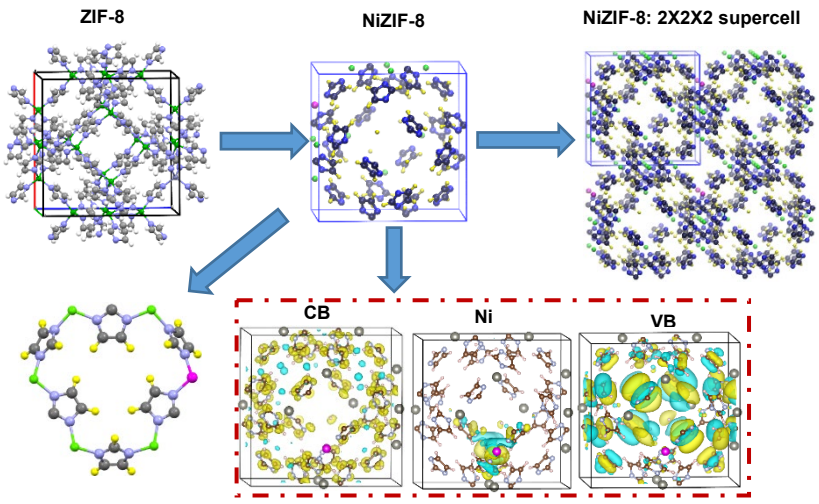

Figure 1. Computational modelling of NiZIF-8 MOF

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Efficiency Evaluation of CRISPR Knock-out model of a Large, Multi-isoform Gene: Overcoming Isoform Complexity and Genomic Size

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Mutations that affect the biogenesis, structure, or function of cilia lead to dysregulated cellular signaling, resulting in a group of disorders known as ciliopathies. These conditions can affect a single organ or multiple systems, as seen in Alström syndrome, a rare disease characterized by retinal dystrophy, obesity, insulin resistance, and sensorineural hearing loss, caused by mutations in the ALMS1 gene.

To investigate the cellular role of ALMS1, we used RPE1 cells, a non-transformed human retinal pigment epithelial cell line commonly used to study primary cilia due to their robust ciliogenesis. ALMS1 is a large and structurally complex gene with multiple isoforms generated through alternative splicing under physiological conditions. To ensure full gene inactivation, we designed a CRISPR-Cas9 knockout model targeting an exon region shared by all known isoforms preventing the production of any functional protein variants.

Two selection strategies were tested to determine the most suitable for RPE1 cells: one using a plasmid carrying puromycin resistance and another expressing GFP. Selection was performed via antibiotic treatment or flow cytometry, respectively. Clones were subsequently analyzed by immunofluorescence to assess protein expression and by qPCR to quantify mRNA levels.

Looking ahead, these ALMS1 KO RPE1 cells will serve as a valuable tool to explore the molecular consequences of ALMS1 loss, particularly in relation to epithelial-to-mesenchymal transition (EMT) and its potential contribution to retinal dystrophy. We plan to analyze the expression of EMT-related genes and proteins by qPCR and Western blotting, aiming to identify dysregulated pathways that might be implicated in retinal degeneration seen in Alström syndrome. This model will allow us to dissect early molecular changes associated with ciliary dysfunction and pave the way for identifying potential therapeutic targets.

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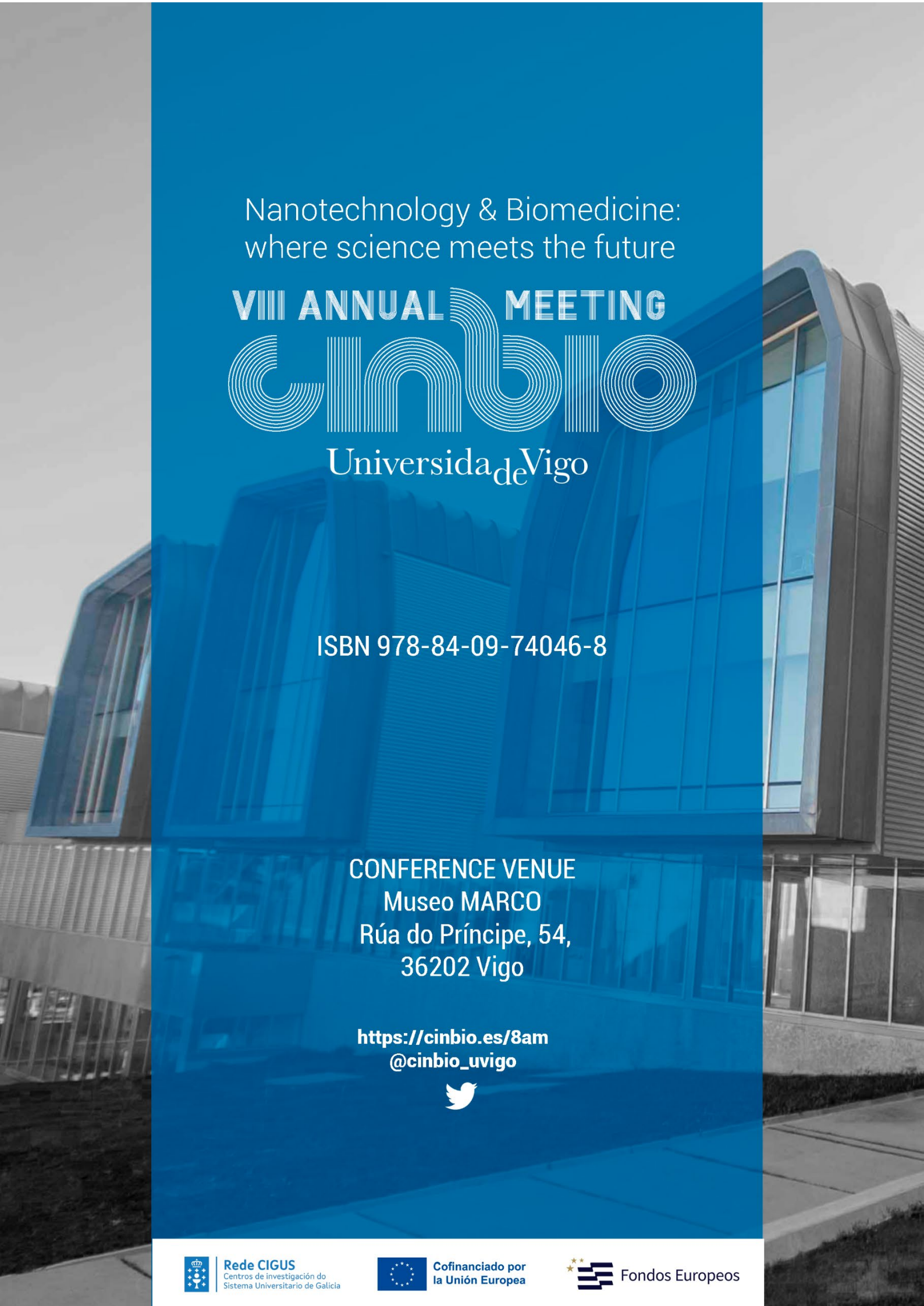

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