Universidade Vigo

28th and 29th July, 2022

# V ANNUAL MEETING CINBIO

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CINBIO Universidade Vigo

Xacobeo 21·22

UNIÓN EUROPEA

XUNTA DE GALICIA

OP ERDF 2014-2020. *"A way to make Europe"*.

V ANNUAL MEETING CINBIO

**Vigo, 28-29 July**

CINBIO presents its V Annual Meeting "Research for life" (28th and 29th, July 2022), organized by CINBIO's postdocs. The V Annual Meeting seeks to bring together researchers interested in chemistry, biology, medicine, physics, mathematics and information technology, showcasing CINBIO's interdisciplinary nature.

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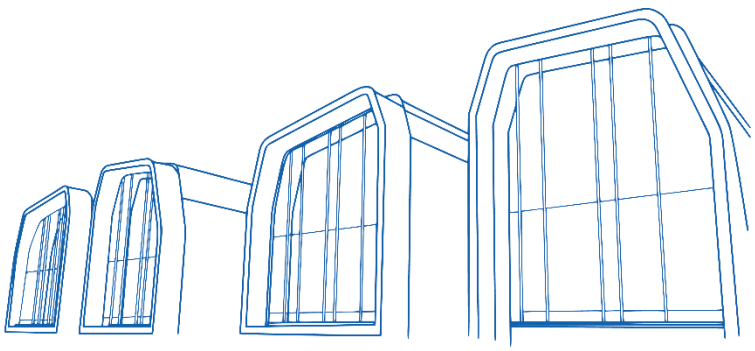

# RESEARCH FOR LIFE

CINBIO Universidade Vigo · Xacobeo 21·22 · UNIÓN EUROPEA · XUNTA DE GALICIA

OP ERDF 2014-2020. *"A way to make Europe"*.

In 2017, CINBIO´s Postdoctoral Researchers began hosting meetings that joined scientists from all research areas covered in the center, with the goal of fostering interdisciplinary dialogue and promoting excellence in research extending beyond traditional disciplinary boundaries.

These are the proceedings for the latest of these meetings, the 5th CINBIO ANNUAL MEETING, celebrated in Vigo, Spain, on July 28th-29th 2022. After having an online edition in 2021, as a natural response to the global challenges posed by the COVID-19 pandemic, we were happy to host this fifth edition of the Annual Meeting again as an in-person event.

This call to scientific discussion was heeded by more than 150 participants, including 2 keynote speakers, 8 invited speakers, 12 oral presentations, and 55 posters. Although these proceeding do certainly not capture the interesting discussions triggered by these presentations, I expect that they serve as both reminder and bookmark of the ideas that you found exciting within them.

It was a pleasure having visitors and colleagues enliven the institute by sharing their work with us and engaging in deep conversation at the Q&As, social breaks, and poster sessions. I am thankful of your contribution to the success of this event, and I hope that your experience was as good as mine, leaving new ideas alongside good memories.

Thank you all for contributing with your ideas and presentations, the lifeblood of this event, and thank you as well to the organizing and scientific committees for providing the structure for this organism.

I cannot wait for the sixth edition of CINBIO Annual Meeting, in which I hope to find you again.

Warmly,

Miguel Ángel Correa Duarte

Director of CINBIO

PROGRAM

## 28th July

9:00 - 9:30 Registration
9:30 - 10:00 Opening and Welcome.

### SESSION 1: Chairs - Ana Sousa, Sergio Rodal

**10:00 - 10:45 (KS1) Aitziber López Cortajarena**
Biomolecular Nanotecnology group of CIC-BiomaGUNE, Donostia
*Protein-nanomaterial engineered composites: a new horizon for biologic drugs and diagnostic tools*
**10:45 - 11:15 (IS01) Riccardo Marin**
Nanomaterials for Bioimaging Group (nanoBIG), Universidad Autónoma de Madrid
*Silver sulfide nanocrystals for imaging and sensing: a Bildungsroman*
**11:15 - 11:30 (ST01) María Relvas**
International Iberian Nanotechnology Laboratory (INL), Braga
*Remote cancer monitoring using Surface Enhanced Raman Scattering (SERS) Spectroscopy Technology*
11:30 - 12:15 Coffee & Posters

### SESSION 2: Chairs - Paula Lorenzo, María Blanco

**12:15 - 12:45 (IS2) Ramón Reig**
Instituto de Neurociencias CSIC-UMH, Alicante
*Synchronization of multisensory information in dorsomedial striatum*
**12:45 - 13:15 (IS3) David Brea**
FCT Researcher Assistant at Champalimaud Foundation, Lisboa
*Brain-immune interactions in stroke: a two-way pathway*
**13:15 - 13:30 (ST02) Daniel Rodríguez**
Department of Chemistry, CICA & Faculty of Sciences, Universidade da Coruña
*Enzymatic activity, structure, and inhibition of human myeloperoxidase: an experimental and computational approach*
**13:30 - 13:45 (ST03) Uxía Gómez**
Departamento de Química Orgánica y Instituto de Investigación Sanitaria Galicia Sur (IISGS), Universidade de Vigo
*Analogues of vitamin D and cancer therapy: a new opportunity*
13:45 - 15:30 Lunch & Networking

### SESSION 3: Chairs - María Xosé Rodríguez, Hugo López

**15:30 - 16:00 (IS4) Natalia Vilor-Tejedor**
BarcelonaBeta Brain Research Center, Barcelona
*A random walk down in Imaging Genetic studies*
**16:00 - 16:15 (ST04) Marta Cousido**
Instituto Español de Oceanografía (IEO-CSIC), Centro Oceanográfico de Vigo & SiDOR Research Group, CINBIO, Universidade de Vigo
*TwoSampleTest.HD: An R package for the two-sample problem with high-dimensional data*
**16:15 - 16:30 (ST05) Marta Aranda**
International Iberian Nanotechnology Laboratory (INL), Braga
*Continuous remote monitoring of prostate cancer metabolites through an implanted biosensor*
16:30 - 17:00 Coffee & Posters

**SESSION 4: Chairs - Marga Vázquez, Sara Núñez**

**17:00 - 17:30 (IS5) Gil Markovich**
School of Chemistry, Tel Aviv University
*Mechanistic study of symmetry breaking in the formation of chiral nanocrystals*

**17:30 -17:45 (ST06) Juan José Tarrio**
Departamento de Química Orgánica/CIQUS, Universidade de Santiago de Compostela
*Evaluation of substitution effects in the propierties of chiral helical poly(diphenylacetylene)s*

**17:45 -18:00 (ST07) Carla Estévez**
CINBIO, Universidade de Vigo
*Studying the limit of detection of strongly coupled Au@Ag@mSiO2 nanorattles and J-aggregates SERS tags*

## 29th July

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**9:30 - 10:15 (KS2) Alexander Govorov**
Physics and Astronomy Department, Ohio University
*Plasmonic Metastructures and Bio-Assemblies for Optics and Photochemistry: Chirality, DNA-origami, and hot electrons*

**10:15 - 10:45 (IS6) María Tomás**
METBioCat group; CIQUS, Universidade de Santiago de Compostela
*Conducting chemistry in living cells*

**10:45 - 11:00 (ST08) Patricia Taladriz**
International Iberian Nanotechnology Laboratory (INL), Braga
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**11:00 - 11:15 (ST09) Ana Costa-Ribeiro**
International Iberian Nanotechnology Laboratory (INL), Braga
*Evaluation of Covalent Organic Frameworks for the development of a low-cost, rapid detection of Shiga Toxin-producing Escherichia coli in ready-to-eat salads*

**11:15 - 11:30 (ST10) Lara González**
CINBIO, Universidade de Vigo
*Surface Enhanced Raman Spectroscopy (SERS)-based Lateral flow immunoassay for ultrasensitive detection of SARS-CoV2*

11:30 - 12:00 Coffee & Posters

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Molecular Evolution Department, Centro de Astrobiología (INTA-CSIC), Madrid
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**12:30 - 12:45 (ST11) Lara Costas**
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Centro de Ciencias do Mar, Facultade de Ciencias y Tecnología, Universidade do Algarve
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13:30 - 14:00 Prizes and closing remarks

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# Keynote Speakers

# Protein-nanomaterial engineered composites: a new horizon for biologic drugs and diagnostic tools

Aitziber L. Cortajarena[1,2]

(1) Center for Cooperative Research in Biomaterials (CICbiomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia-San Sebastián 20014, Spain

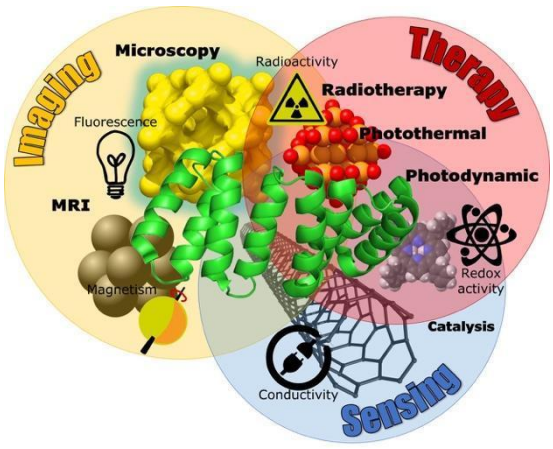
(2) Ikerbasque, Basque Foundation for Science, 48009 Bilbao, Spain.

*alcortajarena@cicbiomagune.es*

The treatment and diagnosis of many diseases still remain a challenge. Inspired by nature, we explore biomolecules and their derivatives as novel therapeutic/diagnostic agents. Among biomolecules, proteins rise huge interest due to their high versatility, biocompatibility, and biodegradability. In particular, we use a class of engineered repeat proteins, the consensus tetratricopeptide repeat (CTPR) proteins due to their stability and robustness as a base scaffold that can be easily tailored to endow desired functions to the protein. For example, the introduction of metal-binding residues (e.g., histidines, cysteines) drives the coordination of metal ions and the subsequent formation of nanomaterials.[1] Additionally, new binding capabilities can be encoded within the CTPR unit or this can be conjugated with other peptides/proteins.[2] These properties allow the development of protein-nanomaterial composites.[2,3] Generally, the fusion of two distinct materials exploits the best properties of each, however, in protein-nanomaterial composites, the fusion takes on a new dimension as new properties arise.

These composites have ushered the use of protein-based nanomaterials as biopharmaceuticals beyond their original therapeutic scope and paved the way for their use as theranostic agents. In this context, engineered proteins have emerged as promising scaffolds to hold simultaneously therapeutic and diagnostic functions, as has been recently demonstrated in our pioneering in vitro and in vivo examples.[2,3]

*Scheme of engineered protein-nanomaterial composites and potential applications.*

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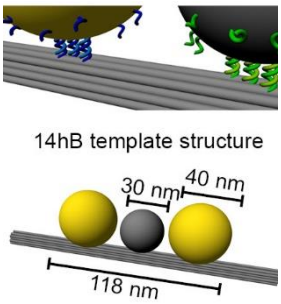
# Plasmonic Metastructures and Bio-Assemblies for Optics and Photochemistry: Chirality, DNA-origami, and hot electrons

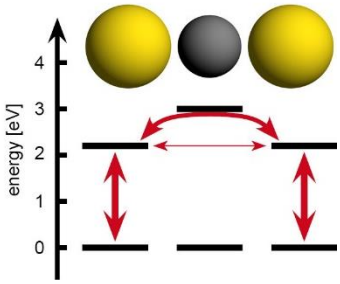
Govorov, Alexander O.[1,*]

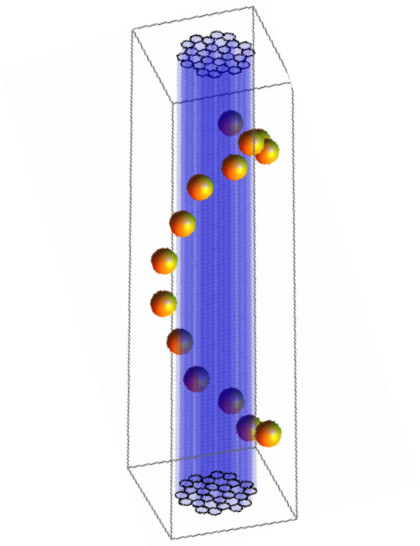
(1) Department of Physics and Astronomy, Ohio University, Athens, USA

**corresponding author: govorov@ohio.edu*

Plasmonic nanostructures and metamaterials are very efficient at the absorption and scattering of light. The studies to be presented in this talk concern special designs of hybrid nanostructures with electromagnetic hot spots, where the electromagnetic field becomes strongly enhanced and spatially concentrated. Overall, plasmonic nanostructures with hot spots demonstrate strongly amplified optical and energy-related effects, and this talk will review some of such phenomena. (1) Using nanoparticle arrays made of different metals, one can transfer plasmonic signals coherently and with minimal losses [1]. (2) Plasmonic hot spots efficiently generate energetic electrons, which can be used for photochemistry and photodetection [2,3,4]. (3) Nanostructures with small interparticle gaps can strongly enhance heat's optical generation and confine high photo-temperatures in small volumes [5,6,7]. (4) Colloidal nanocrystal assemblies and metastructures with plasmon resonances allow us to strongly enhance the chiral optical responses (circular dichroism) of biomolecules and to induce chiral photo-chemical effects [8,9,10,11].



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# Invited Speakers

# Silver sulfide nanocrystals for imaging and sensing: A *Bildungsroman*.

Marin, Riccardo[1,*]

(1) Nanomaterials for Bioimaging Group (nanoBIG), Departamento de Física de Materiales, Facultad de Ciencias, Universidad Autónoma de Madrid, C/ Francisco Tomás y Valiente 7, Madrid 28049, Spain.

**corresponding author: riccardo.marin@uam.es*

Fluorescence imaging affords real-time images of biological tissues in a safe, non-invasive way thanks to the use of non-ionizing radiation (i.e., photons) both as the probe and the collected signal. The use of photons translates also to the need for a relatively inexpensive imaging setup that is easy to operate – being composed, in its simplest form, of a laser source and a photodetector. This is in stark contrast with established methods like computed tomography, magnetic resonance, and positron emission tomography, which require expensive and cumbersome equipment, sometimes employing ionizing radiation. Although fluorescence imaging could rely on the autofluorescence of endogenous tissue components, the use of luminescent species as contrast agents greatly improves the performance of this imaging technique. In this vein, luminescent nanoparticles have emerged as ideal candidates to act as fluorescence imaging contrast agents. Among them, silver sulfide nanocrystals have recently become the prime choice, owing to their benign chemical composition, near-infrared emission, and brightness. Moreover, their sensitiveness to temperature changes enables gathering information about the thermal state of biological systems. In this talk, silver sulfide nanocrystals will be presented as one of the most promising contrast agents for fluorescence imaging. After discussing synthesis strategies and methods to increase the brightness of these nanocrystals, their applications at the preclinical level will be presented. Challenges lying ahead and possible research directions will also be highlighted.

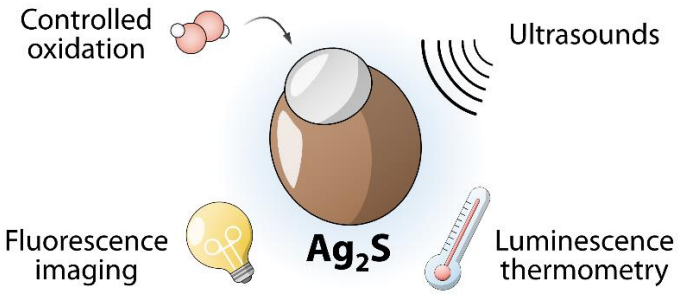


***Figure.*** *Ways to improve and uses of $Ag_2S$ semiconductor nanocrystal in biomedicine*

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[5] R. Marin et al, Adv. Photonics Mater., 3, 2100260 (2022)

# Synchronization of multisensory information in dorsomedial striatum

Reig, Ramón[1,*]

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How the brain operates with different sensory discrepancies is an essential question in order to understand how visual and somatosensory perturbations engage motor responses. The basal ganglia (BG) are involved in motor functions. The striatum is the input layer of the BG and receives cortical projections from sensory, motor and associative areas and thalamus. The rodent's dorsal striatum is functionally subdivided in two regions; the lateral and medial striatum (Alegre-Cortes et al. 2021). The 95% of the striatal neurons are GABAergic projection neurons called MSNs. They are divided into two subpopulations according to their axonal projections and their different dopamine receptor expression, defining the direct and indirect pathway. In addition, the striatum is massively innervated by dopaminergic axons from the substantia nigra pars compacta. Dopamine is known to play a role in processes leading to corticostriatal synapses, modulating and inducing changes in synaptic transmission and plasticity. Single neurons in the dorsomedial striatum (DMS) are excited by tactile and visual inputs, thus processing information from different modalities. We studied how dopamine modulates the integration of this bimodal information in mouse DMS, as well as its effect on spontaneous activity. We obtained *in vivo* whole-cell recordings from identified medium spiny neurons (MSNs) during presentation of tactile, visual and simultaneous bimodal stimuli in resting conditions or after DA-induced release. Our results show that DA induces the synchronization of bimodal information specifically in direct pathway MSNs Altogether, our results propose a new mechanism underlying multisensory integration mediated by DA release.

# Brain-immune interactions in stroke: a two-way pathway

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Stroke is a “brain attack”, that occurs when blood flow to an area of the brain is interrupted. When this happens, brain cells are deprived of oxygen/glucose and begin to die. When brain cells die, abilities controlled by that are of the brain such as memory, speech, or motor control are lost. Stroke pathophysiology involves a series of complex events including excitotoxicity, calcium overload, oxidative stress, and apoptosis. These mechanisms trigger an important immune/inflammatory reaction, characterized by the activation of microglia (immune cell resident in the brain), the release of cytokines and the infiltration of peripheral immune cells.

An important source of immune cells may be the intestinal compartment, one of the largest immune organs in the body, where immune cells are “educated” by the continuous interaction with commensal microbes. Therefore, we investigated the effects of intestinal flora on the immune system and the outcome after cerebral ischemia. We were able to show a novel microbiota-immune-brain axis that is based on the observation that the alteration of intestinal microbiota (dysbiosis) induces a profound remodeling on the intestinal immune system. This remodeling is characterized by the expansion of an anti-inflammatory cell subtype, namely Regulatory T cells, and the suppression of a pro-inflammatory cell subtype, namely IL-17+ γδ T cell. Our data suggest that, after stroke, T cells are able to traffic from the intestine to the Central Nervous System,[1,2] localized in the meninges and increase neuroinflammation by secreting IL-17. IL17 is a proinflammatory cytokine that triggers the production of other mediators resulting in the infiltration of additional immune cells with a cytotoxic phenotype, and contributing, in this way to the stroke pathophysiology[1]

Based on these data, one could expect that anti-inflammatory/immunomodulatory therapies may be a good option for stroke treatment. However, so far, inhibition of inflammatory response (for example with IL-1R blockers), blocking leukocyte infiltration (for example with natalizumab), or treatments with pleiotropic immunomodulators (eg, allogeneic adult stem cells) have been unsuccessful on treating stroke in patients. Part of these failures is because stroked brain communicates with the peripheral immune system inducing what is called post-stroke immunosuppression. This immunosuppressive response of peripheral immunity contributes to post-stroke infections and other important secondary effects that will be reviewed during this talk and that compromise stroke outcome at short and long term.

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# A random walk down in Imaging Genetic studies

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The complexity of neurodegenerative diseases is not only apparent from their clinical presentation, but it is also corroborated by a range of preclinical and experimental studies. To identify which underlying molecular pathways are relevant in neurodegenerative processes, big data has been successfully exploited leading to many insights into their pathophysiology. For instance, the study of genetic factors and their combined action with brain features can help us to determine biological mechanisms in stages prior to the appearance of symptomatology. Specifically, there has been a particular interest in the integrative analysis of genetic data with neuroimaging information (framework known as “imaging genetics”), which represents a current advance for the development and improvement of diagnosis and personalized medicine of complex neurological diseases (Medland et al., 2022). Polygenic risk scores (PRS) are extremely useful in this context. PRSs combine the individual effect of each genetic variant in a single score that summarizes the genetic predisposition of each individual to a specific disorder/condition. The quantification of genetic scores will be useful to explore the association between the elevated genetic predisposition of a certain disease and candidate brain features related to neurodegeneration, in people without traditional risk factors. This strategy will help to further understand biological processes related to individual genetic predisposition, years before neurological symptoms appear, and thus will help to promote preventive practices and interventions. Another strategy consists of analysing both multivariate neuroimaging and genetic data to better capture the complex relationships that may exist between different biological levels. An example of this strategy is the application of the multiple factorial analysis and its extensions (Vilor-Tejedor et al., 2019). The inclusion of the multivariate perspective provides an improvement in the statistical power and predictive capacity in IG studies. Finally, novel work is focused on considering whether the genetic predisposition to complex disorders is associated with the joint modulation of specific brain features (Vilor-Tejedor, et al., 2021).

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# Mechanistic study of symmetry breaking in the formation of chiral nanocrystals

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The handedness of chiral $TbPO_4 \cdot H2O$ nanocrystals can be controlled by preparing the nanocrystals in the presence of certain natural chiral acids, such as tartaric acid. We use circularly polarized luminescence measurements of $Eu^{3+}$ dopant ions in the nanocrystals to follow the handedness and enantiomeric purity of the produced nanocrystals.[1] Using single particle circularly polarized luminescence microscopy we were able to determine the handedness of individual nanocrystals and confirmed that we obtain enantiomerically pure terbium phosphate nanocrystals when prepared with enantiopure tartaric acid molecules.[2] In addition, we explored the influence of the enantiomeric purity of the formed nanocrystals under different conditions, such as synthesis temperature. We find that at low enough synthesis temperatures the nanocrystals break symmetry with tiny impurities and propose a model for the symmetry breaking effects in the nanocrystal formation. We have also studied the formation mechanism of the nanocrystals by in-situ monitoring of their emission, and found that they grow via formation of pre-nucleation clusters.[3]

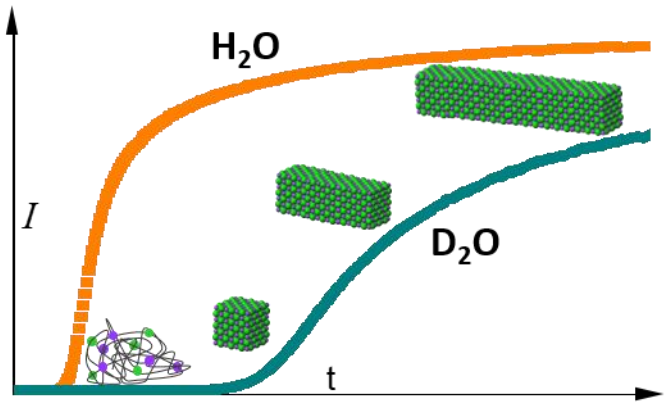


***Figure.*** *Nanocrystal formation kinetics mechanism followed by $Eu^{3+}$ emission intensity as a function of time, exhibiting a large isotope effect between $H_2O$ and $D_2O$ (ref. [3]).*

*This work was supported by the Israel Science Foundation.*

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# Conducting chemistry in living cells

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Bioorthogonal chemistry has revolutioned the way that chemists approach natural systems in order to understand and interfere in the biological processes in their native context.[1] Thus, it is highly desirable to expand the palette of bioorthogonal transformations than can be performed inside living cells. In this context, organometallic chemistry has emerged, in the last years, as an attractive approach to promote artificial reactions in native settings.[2] Nowadays, the development of new processes that respond to external stimuli is attracting interest since they allow to perform chemistry on demand: only when the stimulus is applied the reaction is triggered.

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# Protein microarrays for a highly sensible detection of anti-SARS-CoV-2 antibodies in serum

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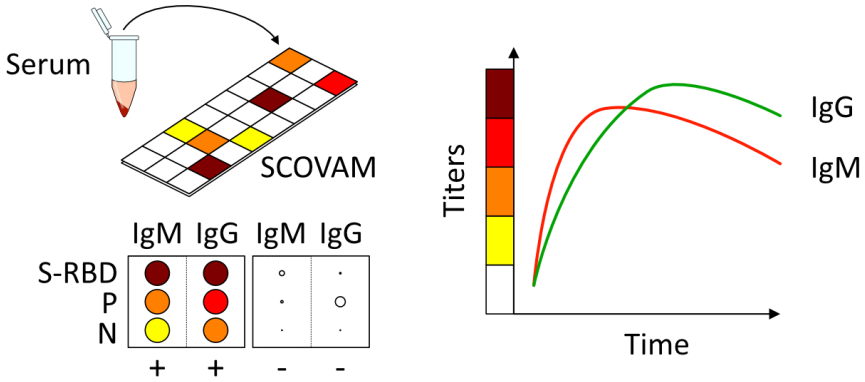
Protein microarrays are a tool to identify target molecules on a solid surface. In our group we have ample expertise in the analysis of environmental samples with this technique using SOLID, an instrument that can process regolith and detect whether bacterial strains, specific proteins and/or small molecules are present. During the Covid19 pandemic, we have applied this knowledge to the development of SCOVAM, a fluorescence-linked immunosorbent assay with several viral antigens (spike, nucleocapsid, main protease Nsp5) of SARS-CoV-2 as capturing probes in a fluorescence immunoassay for COVID-19 serological testing. Given its specificity and sensitivity, we will further apply this technology to monitor the health status of astronauts during spaceflight, which suffer harsh living conditions due to stress, radiation, or a closed environment.




*Blood serum was tested for the detection of specific IgM and IgG antibodies against key SARS-CoV-2 proteins. The antibody titers were followed through time.*

*This work has been funded by INTA's internal budget, the Spanish Ministry of Science and Innovation grant nos. RTI2018-094368-B-I00, and MDM-2017-0737 (Excelencia 'María de Maeztu' to Centro de Astrobiología), the 'Programa de Atracción de Talento' of the local Government of Madrid and the CSIC project number 202020E079.*

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# The potential of inhalation in therapeutics

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Pulmonary drug delivery is gathering considerable attention nowadays, benefiting from the fact that the lung may be used as a port of entry of drugs aimed at either local or systemic effect. On one side, lung delivery of drugs is attractive to address local lung diseases, which is currently the major application of the delivery route. In this context, apart from conventional approaches on asthma or chronic obstructive pulmonary disease (COPD), other respiratory diseases are now seeing scientific efforts translating into therapeutic solutions that involve drug inhalation, such as cystic fibrosis, tuberculosis, pneumonia or lung cancer. The possibility to concentrate the drug where needed, providing the co-localisation of drugs and action sites, with the consequent decreased systemic exposure and diminished side-effects, is certainly beneficious in this regard. On the other side, the lung also displays anatomical and structural characteristics that potentiate the use of this route for the delivery of systemically acting drugs. Such beneficial characteristics include the large surface area (~100 $m^2$) that is available for drug absorption, high vascularisation and low metabolic activity comparing with other routes of delivery [1]. Some biopharmaceutical drugs are now finding their application through inhalation. In parallel to promising therapeutic approaches, the lung route may also play a role in immunisation strategies, which is of particular interest for immunisation against airborne diseases [2]. Regardless the purpose, deliver drugs/antigens to or through the lung implies working out a formulation complying with specific requisites to reach the desired site of action. Therefore, formulation is key in the development of successful lung drug delivery strategies.

In this lecture, the beneficial properties of the lung for drug delivery applications will be presented, along with the challenging characteristics that drive the design of suitable inhalable drug carriers. Potential applications of this route for the treatment/prevention of respiratory diseases will be highlighted, with particular focus on tuberculosis therapy and immunisation strategies. In the end, the audience is expected to deepen the knowledge on the abilities of the lung route for the delivery of drugs, be conscious about the inherent challenges and aware of the potential of inhalable therapies.

*Funding from the Portuguese Foundation for Science and Technology (UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020) is acknowledged.*

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# Short Talks

# Remote cancer monitoring using Surface Enhanced Raman Scattering (SERS) Spectroscopy Technology

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Remote patient monitoring of cancer diseases can potentially increase current predictive rates, while contributing to a more cost-effective and accessible diagnosis and treatment. Patients at high risk of cancer recurrence constitute an ideal population for such improved cancer monitoring tools. These novel tools should have the ability to remotely monitor patient data, which can be used to detect disease onset or progression [1].

Herein, it is proposed to use biomaterials, nanotechnology and machine learning to monitor high-risk profile cancer patients. The technology is based on a minimally invasive and biocompatible implantable biosensor. For this, a hydrogel matrix was combined with plasmonic nanoparticles (gold nanostars - GNS) [2, 3] to act as biomarker signal enhancers through surface-enhanced Raman scattering (SERS) spectroscopy. SERS is a powerful and highly sensitive analytical technique with growing applications in the medical field [4].

For the optimization and validation of the biosensor, different concentrations of GNSs as well as hydrogel formulations were tested. The hybrid material was optically and morphologically characterized with UV-Vis-NIR spectrometry, transmission electron microscopy and Raman and SERS spectroscopies. To evaluate the SERS efficiency of the novel biosensor different chemical and biological compounds were tested [5]. Furthermore, due to the intended intradermal placement of the biosensor, sterilization strategies were evaluated for the GNS solutions and for the hydrogel material.

*Acknowledgements*

*SENTINEL –Novel injectable biosensor for continuous remote monitoring of cancer patients at high-risk of relapse (Projeto45914 -04/SI/2019, funded by Programa Operacional Regional do Norte –Norte 2020)*

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# Enzymatic activity, structure, and inhibition of human myeloperoxidase: an experimental and computational approach

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The enzyme myeloperoxidase (MPO) is the most abundant protein in neutrophils. It plays a key role in immune system in mammals, catalysing the formation of the strong oxidizing and chlorinating agent hypochlorous acid (HClO), the most reactive two-electron oxidant produced in human body. Thus, MPO is actively involved in the protection against exogenous microorganisms and represents a significant factor in the development of numerous disorders related to inflammation: several types of cancer, neurodegenerative diseases, arthritis, etc.

There are numerous studies on myeloperoxidase, particularly from a clinical or biochemical point of view, but the precise reaction mechanism of the chlorination process catalysed by the enzyme was still unknown. However, a detailed knowledge of the structure and reactivity of myeloperoxidase, its intermediates and substrates, is essential for a comprehensive understanding of the enzymatic activity and the development of treatments and drugs.

The active site of MPO is hidden deep inside the protein, connected to the outer medium through a narrow substrate channel. The exhaustive reaction mechanism has been determined by kinetic studies,[1] making use of this particular arrangement, which allowed us to establish that the formation of both a free oxidizing agent (HClO) and an enzymatic chlorinating species takes place.[2] Pulse radiolysis experiments have shown the essential shielding role of the peptide surrounding the active site.[3] The detailed structures of different enzymatic complexes have been worked out with computational calculations. Further research is currently being conducted for the rational design of more effective MPO inhibitors.

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# Analogues of vitamin D and cancer therapy: a new opportunity

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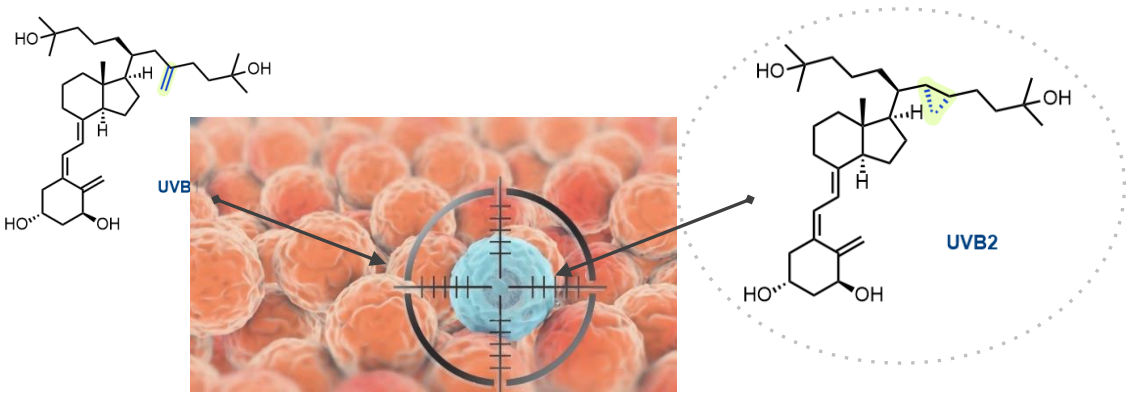
Colorectal and breast cancer are the most commonly diagnosed types of cancer and the leading cause of cancer mortality. In spite of improved by treatment with chemotherapy, it is necessary to find new targeted therapies that block the growth and spread of these cancer types. Several ongoing efforts are being made to develop novel drugs to treat this pathology with the aim to overcome resistance.

The active form of vitamin D has shown to have antiproliferative, pro-apoptotic, anti-cell migration and antiangiogenic activity in number of preclinical studies in many different cancer types. The research focuses on the rational design of new analogues to define more potent compounds and hoping to reduce the propensity to cause toxic hypercalcemia effects.[1]

In fact, we synthesized a new type of Gemini vitamin D analogue named **UVB1** which has potent antitumoral effects over a wide range of tumor cell lines and lacks hypercalcemic activity. The results obtained reinforce its potential use as antitumor agent to treat colorectal and breast carcinomas.[2]

Recently, a novel analogue of **UVB1** has been designed in order to induce important improvements in its behavior as biological ligand. This new analogue (**UVB2**) shows additional chiral centers which is a huge challenge in performing its total synthesis.

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# TwoSampleTest.HD: An R package for the two-sample problem with high-dimensional data

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Nowadays high-dimensional data is a recurring theme within Statistics. The main property of the high-dimensional setting is that the number of variables, or locations, is large, while the sample size is usually small. This type of high-dim, low sample size data arises in many different areas of science, such as genetics, medicine, pharmacy and social sciences. With microarray data, for example, the variables typically represent the expression levels of a large set of genes for a small number of individuals. In this work we introduce the new R package TwoSampleTest.HD for such a setting. The focus is on testing for the null hypothesis that the marginal distributions of the large set of variables are the same for two groups of individuals. For that aim, TwoSampleTest.HD package implements the tests recently proposed by Cousido-Rocha et al. (2019) for the low sample size, high-dim setting taking the possible dependence among the variables into account. The fact that the tests are adapted for dependence is a crucial feature since, in the majority of examples with high-dimensional data, the target variables are not independent. Specifically, the tests implemented in TwoSampleTest.HD are based on the distance between the empirical characteristic functions of the two samples, when averaged along the variables or locations. Different options to estimate the variance of the test statistic under dependence are allowed. The package TwoSampleTest.HD provides the user with individual permutation p-values too, so feature discovery is possible when the null hypothesis of equal distribution is rejected. We illustrate the usage of the package through the analysis of a large number of gene expression levels measured on two groups of patients with breast cancer. The objective is to test for the null hypothesis that the distribution of each of the genes is the same for the two tumor types and, if the null is rejected, to identify the genes which are differently expressed. Additionally, the package is illustrated from simulated data in order to highlight the power improvements of the methods implemented in TwoSampleTest.HD over other well-known approaches such as Kolmogorov-Smirnov-based multiple testing. Practical recommendations are given.

*The authors acknowledge financial support from the Grant PID2020-118101GB-I00, Ministerio de Ciencia e Innovación.*

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# Continuous remote monitoring of prostate cancer metabolites through an implanted biosensor

Aranda Palomer, M.[1], Relvas, M.[1], Hashemi, M.[2], King, JB.[2], Chen, M.[2], Tunnell, JW.[2], Cautela, M.[2], Costa, P.[2], Learmonth, D.[2], Sousa, R.[2], Carbó-Argibay, E.[1], Diéguez, L.[1], Abalde-Cela, S.[1]

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The development of novel monitoring tools for continuous remote cancer progression may be used to detect cancer relapses in post-operative or post-treated patients. [1] Herein, we present an implantable biosensor to be applied in the context of prostate cancer diagnosis and monitoring of relapse, through surface-enhanced Raman scattering (SERS) spectroscopy. SERS is an ultrasensitive technique with many applications in the biomedical field, offering high selectivity and sensitivity, multiplexing capabilities and label-free detection. [2] This biosensor will be implanted intradermally to monitor the signals of the interstitial fluid (ISF) metabolites upon diffusion into the injected biosensor. A highly controlled data analysis workflow is being developed in parallel to normalise signals acquired through different skin types and from a complex biological matrix. As such, a machine learning algorithm is being developed and optimised to classify the patients according to the recorded SERS patterns in a label-free manner. Initially, the biosensor was tested in artificial ISF to first have a library of the expected Raman signals, and then, human biofluids were incubated in the biosensor to test the signal acquisition from cell metabolites. In recent results, some cancer biomolecules were detected by SERS, after to test the biosensor in cellular supernatants from prostate cancer cell lines.

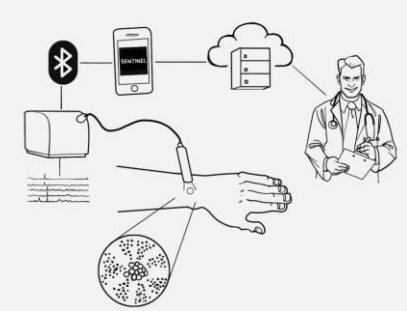

***Figure 1:*** *A biosensor to monitor cancer in patients by a handheld Raman device.*

*This project is supported under the framework of the Incentive Scheme to Research And Technological Development (Si I&Dt), Corporate R&D – Copromotion Projects: International Partnerships.*

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# Evaluation of substitution effects in the properties of chiral helical poly(diphenylacetylene)s

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Helical structures are some of the most relevant motifs present in Nature. Proteins, nucleic acids or polysaccharides are biomolecules whose structures are formed by helices. These secondary structures are directly related to the properties of those molecules. For this reason, the scientific community has invested time and effort in obtaining new molecules that can adopt the helical structure in order to emulate the functions and properties of these biomolecules.

Dynamic helical polymers have been widely studied due to the potential of these materials to act as sensors, chiral stationary phases for chromatographic separations or chiral catalysts because of their helical sense control via external stimuli.[1]

In order to find new structures and new functions in this field, this work addressed the synthesis of a novel family of helical polymers denominated poly(diphenylacetylene)s (PDPAs). These polymers are a special class of conjugated polymers that present the abilities of fluorescence emission and a high thermal stability.[2]

Achiral PDPAs have been studied in the last two decades,[2],[3] but it has not been until recent years that chirality has been introduced into the polymers. Recently, our group has developed a new chiral PDPA and elucidated its secondary structure.[4] In this work, we analyze how the substitution in *para* and *meta* affects the structure and properties of these polymers. These materials have thermo-chiral properties induced by high temperature solvent annealing and fluorescence emission due to the high conjugation of phenyl rings and the polyene backbone. The combination of these two properties makes these types of polymers promising materials for Circularly Polarized Luminescence (CPL) applications.

*Acknowledgement*

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# Studying the limit of detection of strongly coupled Au@Ag@mSiO$_2$ nanorattles and J-aggregates SERS tags

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Strong coupling is a resonant interaction that could happen between a confined electromagnetic field and a molecular transition. This phenomenon occurs when the energy transfer between the light and the molecular transition is larger than their average dissipation. This leads to the formation of a matter-light hybrid state (quasi-particle) whose properties are different from the original constituents [1,2]. Strong coupling regime can be achieved, for example, between plasmonic resonances in metal nanoparticles and excitonic transitions, named plexcitons. Recently, publications have evaluated the use of plexcitons in applications for energy collection, catalysis, or optical communications [1]. In this work, we move forward studying how plexcitonic nanoparticles obtained from the strong coupling of plasmonic nanoparticles and J-aggregates can be used as ultra-efficient SERS nanoprobes.

Plexcitonic nanoparticles can be obtained from the deposition of J-aggregates at the surface of a metallic nanoparticles. However, these new hybrids show limited colloidal stability strongly dependent on the dispersion medium. Therefore in this work, we explore an alternative synthetic route with Au@Ag@mSiO$_2$ nanorattles. The plasmonic nanoparticles are composed by an Au@Ag nanorod inside of a mesoporous silica capsule. While the role of the plasmonic nanoparticle is to interact with the J-aggregate, the mesoporous silica shell would enhance the colloidal stability. These hybrid systems have been obtained by a two-step protocol: (1) diffusion of the monomers of cyanine dyes inside of the nanorattles through the mesoporous shell and (2) J-aggregate formation on the metal surface via solvent exchange. The resulting hybrids have been characterized by UV-vis-NIR spectroscopy, Transmission Electron Microscopy (TEM) and surface-enhanced Raman scattering. Our preliminary results show that strongly coupled SERS tags could achieve limits of detection in NPs/mL two orders of magnitude shorter than weakly coupled ones.

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# Gold nanorods-based bioactuator as dynamic cell substrate

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Static cell substrates models are widely used to investigate cell responses to mechanical changes. However, these models cannot change their properties *in situ*; therefore, there is a limitation on mimicking *in vivo* dynamics. Hydrogel-based stimulus-responsive bioactuators have been introduced to overcome this limitation. Herein, we describe the fabrication of an Au NRs/PDMS-based bioactuator with a mechanical response close to native tissue, which reversibly changes its stiffness under NIR irradiation. The change in stiffness in NIH-3T3 fibroblasts adhesion and proliferation was evaluated after 24 and 48 hours of NIR illumination. Based on our results, these novel photo-responsive films are promising candidates as dynamic cell substrate models.

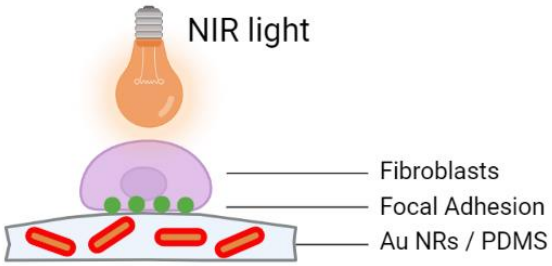


***Figure*** *Gold nanorods (Au NRs) embedded in PDMS are used as photothermal transducers to manipulate fibroblasts response in situ. By exposing the nanocomposite films to a NIR light source, a stiffening effect was achieved, resulting in the clustering of focal adhesions by fibroblasts.*

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# Evaluation of Covalent Organic Frameworks for the development of a low-cost, rapid detection of Shiga Toxin-producing *Escherichia coli* in ready-to-eat salads

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DNA amplification by real-time PCR (qPCR) is a useful tool for foodborne pathogen detection. For successful DNA amplification, it must be concentrated and purified, which is challenging in vegetables due to the presence of inhibitory compounds, such as chlorophylls. Covalent Organic Frameworks (COFs) have been shown to efficiently capture contaminants from aqueous samples [1], and could therefore provide an efficient manner to extract qPCR inhibitors from the sample matrix. The objectives of the current study were three, (i) evaluate different COFs to remove qPCR inhibitory compounds present in vegetable samples, (ii) Develop a multiplex PCR assay for Shiga Toxin-producing *E. coli* (STEC) detection in vegetables and (iii) Evaluate the performance of the combined COF-qPCR in spiked vegetable samples. A total of five different COFs were compared against a reference DNA extraction method. The materials behaved differently, being TpBD-Me2 the one exhibiting the best results in terms of DNA concentration and purity obtained from spiked samples. A pentaplex qPCR was designed, targeting the main virulence genes of STEC, namely, *stx1, stx2* and *eae*, in addition to the *rfbE* gene for the specific detection of the serogroup O157, and an internal amplification control to rule out reaction inhibition. This material was then applied to a panel of 39 spiked ready-to-eat salad samples which were previously inoculated with STEC. The calculated limit of detection for the COF-qPCR was similar to that of the reference method, demonstrating the suitability of this material for the intended application. In addition to this, the methodology demonstrated successful for the detection of cold-stressed STEC.

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# Surface Enhanced Raman Spectroscopy (SERS)-based Lateral flow immunoassay for ultrasensitive detection of SARS-CoV2

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The epidemic of SARS-CoV2 has emerged the necessity for early diagnosis since PoC (Point of Care) iscritical for preventing the spread of the disease. For this aim, we develop a SERS-based LFIA (lateral flowimmunoassay) as an ultrasensitive test for PoC applications employing SERS tags as label probes. SERS is a technique for ultrasensitive chemical analysis that combines the fingerprint molecular information provided by Raman spectroscopy with the enhancement of the plasmonic nanoparticles[1]. The SERS tagsused in this project are based con core-shell gold-silver nanoparticles (Au@Ag NPs)[2] codified with Rhodamine ITC, as Raman reporter molecule, and bioconjugated with antibodies against SARS-CoV2 nucleoprotein (N protein) and spike protein (S protein) to detect the virus in deactivated and activated samples, respectively. Calibration curves for each protein will be carried out by colorimetric and SERS- LFIA[3], with their corresponding limits of detection and quantification, having as final goal the multiplex detection of both antigens. Finally, saliva and nasopharyngeal samples from patients are tested. Besidesin future other virus as coronavirus (OC43), syncytial respiratory or influenza A and B will be investigated.

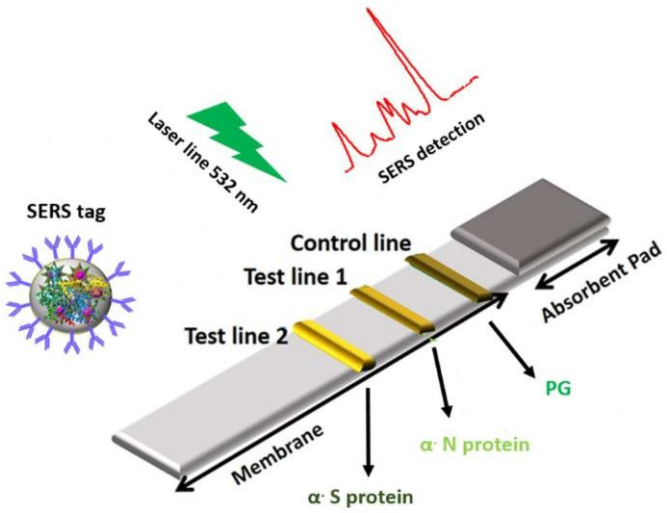


**Figure 1.** Schematic of SERS-based LFIA detection of S and N protein of SARS-CoV2. Only one line isobserved in the control zone in the absence of antigens and three lines in presence of them.

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# Novel *E. coli* biosensors based on Surface enhanced Raman scattering with enhanced multiplexing capabilities

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Advances in synthetic and computational biology improved our capability to fabricate robust bacterial biosensors, making possible the engineering of *E. coli* as a programmable living tool for diagnostic and environmental applications. However, the dependence of current biosensors on bioluminescent, colorimetric, or fluorescent reporters limits the analysis of multiple targets in a single sample. Surface-enhanced Raman scattering (SERS) spectroscopy is an analytical technique that employs plasmonic nanoparticles as optical enhancers for increasing the inherently weak intensity of the Raman signal. The main features of SERS include its high specificity, sensitivity, and multiplexing capabilities owing to the narrow spectral bandwidths that characterise the Raman spectra. In previous studies, we successfully applied SERS for the *in situ* detection and imaging of secreted bacterial metabolites[1,2]. In this work, we evaluated the inducible expression of Raman-active molecules in *E. coli* and, in combination with multivariate statistics, we investigated their potential use as SERS reporters. Our results demonstrate the suitability of the proposed approach, thereby paving the way for a novel class of bacterial biosensors with improved multiplex detection capabilities.

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# EvoPPI 2: an updated version of a powerful Protein-Protein Interaction database aggregator and analysis platform

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Protein interactions determine many cellular processes such as transcription, replication, signal transduction, protein transport, and cell communications, and thus, are essential to understand the cellular architecture. The number of Protein-Protein Interactions (PPI) experimentally described in the literature, using different high-throughput techniques such as yeast two-hybrid and large scale mass spectroscopy of protein complexes, is a signal of their importance. These PPI are publicly available in several databases, but due to the application of different criteria, they show little overlap. To overcome this issue, we created, in 2008, EvoPPI 1.0, a web platform aggregating PPI data from the main public sources available with integrated analysis capabilities. Within EvoPPI 2 (http://evoppi.i3s.up.pt), we updated all EvoPPI 1.0 databases (currently known as 2018.03) and added new ones. Such update resulted in increments in the number of unique interactions in the range of 6.42%-119.95%, depending on the species. In addition, since interacting protein pairs tend to be evolutionarily conserved, the information available for one species might be used to predict the incompleteness of the network in another one and, therefore, allow the identification of putative missing interactions. Based on this, our new database release (known as 2022.04) also includes "predicted interactomes" for *Homo sapiens, Mus musculus, Caenorhabditis elegans,* and *Drosophila melanogaster*, which were created using either Ensembl or DIOPT Ortholog Finder orthologies/paralogies. Finally, we also published the resources to allow researchers to use their own local EvoPPI 2 instances and create custom databases from the existing ones. This is a very interesting option taking into account that not all available PPI data is present in the main databases. For instance, the main databases does not include PPI observed in patient tissues and mutant animal species, where PPI might be aberrant, despite the fact that several studies have shown that in some diseases this is not the case. This way the user can add new data for any species and from any source database, creating custom interactomes to develop their own studies.

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# Posters

# The two-sample problem under random truncation

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Truncation is a problem that comes up naturally when making experiments because of the way data are collected. This phenomenon can be observed in experiments carried out in different fields such as epidemiology, astronomy, biology or industrial life testing. In particular, left-truncation induces an observational bias so large event times are oversampled and proper corrections are needed. In this work we compare the survival functions of two independent populations from left-truncated data to check whether the time-to-event distributions are the same in both groups. For that purpose, we define the Kolmogorov-Smirnov statistic for left truncated data and we show some of its properties. The Kolmogorov-Smirnov test will be used to compared the times until spontaneous abortion in two groups of women [1]: the ones exposed to coumarin derivatives and the control group. These pregnancy times are left-truncated due to delayed entries into study. The introduced Kolmogorov-Smirnov test will allow us to check if the coumarin derivatives have influence on the pregnancy time until a spontaneous abortion.

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# Predicting attrition in a cohort of very preterm infants: statistical methods and machine learning

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Attrition is one of the most challenging problems for researchers in charge of longitudinal cohorts. A cohort affected with attrition may have the validity of its results questioned, as attrition introduces selection bias if the reason behind it is related to the outcome of interest [1, 2]. Efforts to tackle attrition in longitudinal cohorts have been concentrated in two main actions: prevent its occurrence in the follow-up evaluations and develop statistical methods to alleviate its consequences in data analysis. Longitudinal birth cohorts of high-risk children, like those born very preterm (< 32 weeks of gestation), have an important role in providing a comprehensive assessment of the needs and development of these children across their lifespan. This type of cohort produces valuable scientific evidence that, ultimately, will contribute to improving clinical care, supporting public health decisions, and planning of health and education provision to these children. An early and precise identification of which participants present increased risk for dropping out may provide large benefits. Conventional statistical methods, such as logistic regression, have been the usual choice to predict attrition in cohorts. However, these classical theory-based models are constrained by independence, additivity and linearity assumptions which may oversimplify complex relationships between predictors and outcome variables[3]. The application of machine learning methods may bring advantages over the conventional approaches. We developed predictive models of attrition (non-participation) in Portuguese children participating in the geographically defined Effective Perinatal Intensive Care in Europe (EPICE) cohort applying a conventional regression model and different machine learning methods. We further compared the model's performance and identify the most relevant predictors.

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# Developing an automated pipeline for miRNA-seq data analysis in neuropsychiatric research

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Over the past ten years, microRNAs (miRNAs) have gained attention in the neuropsychiatric research field as potential biomarkers of psychiatric conditions. However, a standard protocol for miRNA-sequencing analysis has not yet been established, slowing progress in the field and limiting the usefulness of *in-silico* results [1]. This situation motivated us to develop a novel pipeline for the analysis of miRNA sequencing data in neuropsychiatric conditions, integrating the results of several third-party tools in a highly reproducible workflow. In this study, we performed an initial test of the usefulness of our new pipeline, named “myBrain-Seq” (https://github.com/sing-group/my-brain-seq), by analyzing data from four recent miRNA-Seq studies on neuropsychiatric conditions. We then compared the myBrain-Seq results with those of the original studies and with an additional reanalysis done using another pipeline [2]. Overall, our pipeline was found to have the best level of replicability, although further analyses using datasets with experimental validation data are required in order to allow obtaining meaningful conclusions on myBrain-Seq’s performance.

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# Nonparametric models in genomics: beyond summaries and correlations in high throughput research

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All but the simplest phenotypes are believed to result from interactions between two or more genes forming complex networks of gene regulation. Sleep is a complex trait known to depend on the system of feedback loops of the circadian clock and on many other genes; however, the main components regulating the phenotype and how they interact remain an unsolved puzzle. Genomic and transcriptomic data may provide part of the answer, but a full account requires a suitable quantitative framework.

Using data from an artificial selection experiment for sleep duration with RNA-seq data acquired each generation we propose a Gaussian Process framework to infer interactions between genes from large scale data. We use a Hierarchical Generalized Linear Model analysis to find genes that have significant shifts in expression, and perform inference between all pairs of these genes using a Multi-Channel Gaussian Process model. Our method not only is considerably more specific than standard correlation metrics but also more sensitive, finding relationships not significant by other methods.

Comparison between the results from (Generalized) Linear Models and Gaussian Processes allow some considerations to be made about the approach to large scale data where the mechanisms are unknown. I discuss some of the perspectives of using nonparametric models that retain the main features of interest and are informative about biological mechanisms.

# Isolation and enrichment of leukemic blasts using microfluidics for the detection of Minimal Residual Disease in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is characterised by the accumulation of immature myeloid progenitors in the bone marrow, interfering with the normal production of blood cells. This type of leukemia is the most common in adults and the response to conventional therapies in older patients is limited [1,2]. Moreover, in disease it is very frequent after treatment patients who achieve complete remission relapse due to the persistence of some residual cells that remain undetectable, a condition called minimal residual disease (MRD) [3]. An earlier and accurate diagnosis of MRD would allow for a more accurate prognosis assessment and a better follow-up of the patients. Nevertheless, conventional technologies used for the diagnosis of AML present several limitations in in MRD condition. In recent years it has been demonstrated that microfluidics is a powerful tool for rare cell isolation [4, 5], being one of the potential options to overcome the sensitivity limitations of conventional tools.

Thus, the main goal of this work was to develop a microfluidic system that promotes the concentration and isolation of leukemic blasts based on positive immune-selection. Devices were tested and optimised using cell lines and spiking experiments prior to patient sample analysis. The performance of the device was compared against the gold standard, flow cytometry, and the genetic content of the cells was analysed by sequencing, to demonstrate the utility of the system for MRD assessment and patient stratification.

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# Electrochemical pH monitoring in cell culture systems

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pH has significant influence on the biochemical and biophysical processes of cells and, in fact, acidification of the extracellular media is an essential indicator of overall metabolic activity. As such, measuring pH provides information about the status of the cell culture and is important for determining the necessary media replacement times. Electrochemical methods are attractive for pH sensing, since electrodes can be made in various sizes and readily integrated into all culture systems, from larger reactors to microscopic organ-chips. Also, readout electronics for electrochemical sensors can be compact and cost-effective.

Here we present a potentiometric pH sensor system for real-time monitoring of cell culture. In our system pH sensing is based on an anodically deposited IrOx electrode [1]. Since performance of such potentiometric system is equally depending on the stability of the reference electrode, we have integrated into the platform a pressure driven microfluidic reference electrode [2] to provide stable and sample independent performance. For practical applications in life-science research we implemented a multiplexed instrument, which would be applied to standard 6-well culture plates. In the multiplexed system the single common reference electrode has distributed junctions into different wells, while each well has their own independent IrOx pH sensor. In ordered to operate the system, we have also developed a controller with 6-channel high-impedance inputs for measurement of electrode potential and built-in a low-pressure controller to drive the flow in the reference electrodes. The low-cost electronics and software are intended to be released as open-source to facilitate the wide adoption of the method.

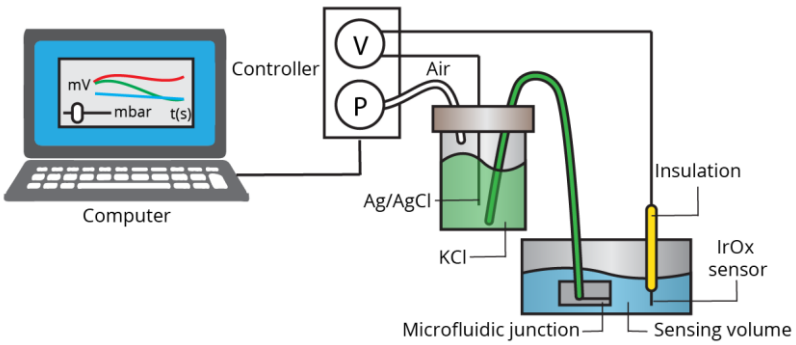


***Figure*** *Schematic illustration of potentiometric pH sensing system showing IrOx sensor and pressure driven reference electrode.*

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# Development of ocular stabilizing and goal-oriented movements in the lamprey

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Our eyes are capable of sophisticated eye movements, evolved through new motor and perceptual needs. The first movements appeared to immobilize the image on the retina: the vestibulo-ocular reflex (VOR), which ensures stabilization of the eyes during head movement, and the optokinetic reflex (OKR), which keeps the visual field sharp on the retina when it is shifted [1, 2]. These reflexes are maintained throughout evolution and date back to the oldest group of living vertebrates; the lamprey [2]. The origin and evolution of the circuits involved are still unknown, however [3]. Lamprey neural circuitry shares many features with other vertebrates, but the connections are simple enough to facilitate their dissection. Such is the case of the neural circuits that transmit information between the vestibular organs and the brain to control gaze and posture [4, 2]. The lamprey's visual system develops stepwise during its larval period. Larvae have immature, skin-covered eyes. Some of the underlying connections to the neural circuits involved in stabilization - mediated by the VOR and the OKR - have been described, but nothing is known functionally [3]. The vestibular system sends its outputs to the extraocular muscles to generate the VOR, but also projects to the spinal cord for producing a compensatory body movement [5]. The optic tectum (OT) is another major visual centre that controls where to direct gaze. In larvae, the OT shows an immature state [3]. The aim is to study when these movements and the neuronal circuits controlling them appear, this includes the circuits controlling VOR and OKR and how these are connected with goal-oriented neuronal circuits such as the OT and its projections to the spinal cord. To achieve this, we studied the connectivity of neuronal circuits with anatomical techniques, electrophysiological recordings and eye tracking to analyse their functionality. Further, we have designed an innovative experimental setup; a platform that allows to generate movement in the three axis while applying visuovestibular stimuli to an *ex vivo* preparation that keeps the nervous system, eyes and vestibular organs. Thus, it was possible to monitor eye movements in response to vestibular and visual stimuli while allowing coordinated electrophysiological recordings. Studying the development of ocular movements provides relevant information to understand the evolution of their underlying neural mechanisms, with implications for all vertebrates.

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# Up-regulation of the dystrophin homolog utrophin via the obestatin/GPR39 system to compensate for the lack of dystrophin in DMD

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Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive neuromuscular disorder caused by mutations in the DMD gene that result in absence of dystrophin, a cytoskeletal protein that enables the strength, stability, and functionality of myofibers [1,2][ (Birnkrant DJ 2018; Blake DJ 2002). The absence of dystrophin leads to progressive muscle weakness, degeneration, and wasting [1](Birnkrant DJ 2018). DMD is the most common form of muscular dystrophy in childhood and there is no cure [3] (Messina S 2018).

Understanding the molecular and cellular mechanisms underlying skeletal muscle regeneration can help to identify new therapeutic approaches to ameliorate significant hallmarks of disorders of skeletal muscle, as DMD. These therapies would activate MuSCs to enhance their regenerative potential, so the dystrophic symptoms would be reduced. In this context, our group has demonstrated that the obestatin/GPR39 system regulates muscle homeostasis acting as an autocrine/paracrine signal, making obestatin a promising therapeutic candidate for the treatment of conditions related to muscle regeneration such as DMD [4] (Gonzalez-Sanchez J 2018). Thus, the specific aim of the present work is to investigate the therapeutic potential of the obestatin/GPR39 system to activate the synthesis of utrophin as focal treatment of dystrophic muscles using in vitro cell culture models of human DMD skeletal muscle cells. We demonstrate that up-regulation of the dystrophin homolog utrophin by the obestatin/GPR39 system correlates with the cooperative activation of the class II HDAC/MEF2 and calcineurin/NFATc1, pathways associated to oxidative phenotype transition.

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# K+ channels and intrinsic electrical properties alterations in a Shank3 mouse model of autism spectrum disorder

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Autism Spectrum Disorders (ASD) are a group of neurodevelopmental alterations arising from a combination of multiple environmental and genetic factors. In the past few years, a strong relation between Shank3 protein mutations and alterations linked to ASD has been established. Patients with alterations in these proteins usually present neural excitability abnormalities. In fact, some studies have found a link between Shank3 mutations and potassium channels dysfunction, but the role of this association on neuronal electrical properties remains unclear. The aim of this work was to study the alterations of the resting membrane potential and the excitability in sympathetic neurons obtained from a Shank3-mutant mouse model of autism. We also investigated the role of background KCNK, KCNQ and HCN channels in those alterations. Using perforated-patch whole-cell patch clamp technique we recorded sympathetic neurons in culture, both in current- and voltage-clamp. When compared with wild type, neurons obtained from Shank3 mutant mice showed a more positive resting membrane potential and a clear altered excitability. Our results show that significant alterations in background potassium channels expression and in currents carried by them are, at least in part, responsible for these variations in neuronal excitability.

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# Tailoring microfluidic immunoassays for detection and characterization of circulating tumour cells in breast, colorectal and lung cancer

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Lung (LC), colorectal (CRC) and breast cancer (BC) are among the deadliest cancers worldwide [1]. Even though they start as a local disease, they often silently metastasize. In fact, metastases are responsible for the high number of cancer-related deaths [2]. Circulating Tumour Cells (CTCs) exhibit great metastatic potential, they can reflect tumour heterogeneity and clonal evolution in real time. They have also proven to be a fundamental tool for early metastasis diagnosis and non-invasive monitoring [3]. However, currently available CTC isolation approaches have suboptimal sensitivity. The RUBYchip™ (PCT/EP2016/078406, licensed to RUBYnanomed) is a novel microfluidic technology for unbiased and efficient CTC isolation and characterization. It has been demonstrated to capture CTCs based on their size and deformability directly from a whole blood sample using different cancer types [4–6]. The standard CTC immunoassay includes the analysis of Cytokeratin and CD45 staining to accurately detect and enumerate CTCs. From the clinical perspective, it is often that different cancer types rely on different key biomarkers for patient stratification and treatment selection, thus in this work we aim to expand and tailor CTC detection immunoassays to include biomarkers such as EGFR, Vimentin and HER2 in the assessment of samples from different cancer types (LC, CRC and BC). Besides immunoassay optimization in experimental samples, 15 clinical samples (including LC, CRC and BC) were used as well for CTC enumeration comparison against the gold standard technology, CellSearch®. Using the RUBYchip™, we were able to identify distinct phenotypic profiles in captured CTCs of different tumour types. This technology allows for differential immunophenotyping in clinical samples, which highlights the biomarker testing versatility of unbiased CTC isolation methods and is expected to contribute towards the implementation of increasingly personalized cancer treatments.

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# Role of voltage-sensitive $Ca^{2+}$ channels in the glufosinate-induced striatal dopamine release in freely moving rats

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**Introduction.** Glufosinate ammonium (GLA) is the ammonium salt of the amino acid phosphinothricin and the active component of a broad-spectrum herbicide used to control weeds in agriculture, domestic areas, and public domains. Given the structural analogy of GLA with glutamate, it is considered that the neurotoxic effects caused by this pesticide could be related to its possible action on the N-methyl-D-aspartate receptors (NMDAR) (Nakaki T et al., Neurosci Lett., vol 290:209-12, 2000). It has been shown in nigrostriatal dopaminergic terminals that GLA increases the dopamine (DA) overflow in freely moving rats in a concentration-dependent way, being this effect partially dependent on extracellular $Ca^{2+}$, since the removal of $Ca^{2+}$ from the perfusion medium significantly decreased the effect of pesticide (Ferreira Nunes BV et al., Arch. Toxicol. 84, 777–785, 2010). Based in this information, in the present study we aimed to investigate the possible involvement of voltage-sensitive calcium channels (VSCC) on the GLA-induced in vivo DA release from rat striatum, using selective N-, and P/Q-type $Ca^{2+}$ channels blockers.

**Methods.** Female Sprague-Dawley adult rats (250-350 g, 5/8 group) were used in the experiments. GLA (10 mM) and the specific VSCC antagonists were administered directly into the striatum through a membrane probe for brain microdialysis. The effects of GLA were observed in presence of two VSCC antagonists: ω-conotoxin MVIIC (20 μM), a P/Q-type antagonist, or ω-conotoxin GVIA (20 μM), a N-type antagonist. The levels of DA, obtained from dialysates, were measured using HPLC-EC. Statistical analysis was made by means of ANOVA and Student-Newman-Keuls test. Significant differences were as follow: $P < 0.05$, $P < 0.01$ and $P < 0.001$.

**Results.** Intrastriatal infusion of GLA significantly increased the DA release to 791 ± 90.1% ($P < 0.001$), compared with basal values. The coinfusion of GLA together with ω-conotoxin MVIIC increased the DA levels to 235.6 ± 79% ($P < 0.01$), with respect to control levels. This result shows that the administration of ω-conotoxin MVIIC significantly reduced the effect of GLA by 70.2% On the other hand, the administration of GLA to animals pretreated with ω-conotoxin GVIA decreased dopamine levels to 31.8 ± 6.1% ($P < 0.05$), compared to baseline levels. This finding shows that ω-conotoxin GVIA completely inhibited the effect of GLA on the DA release from rat striatum.

**Conclusions.** The results obtained in the present study show that GLA-induced DA release could be produced, at least in part, by activation of striatal P/Q- and N-type VSCC located at the synaptic terminals and axons of striatal dopaminergic neurons, especially N-type VSCC.

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# Activation of autophagy in Duchenne muscular dystrophy via the obestatin/GPR39 system

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Duchenne muscular dystrophy (DMD) is an X-linked recessive lethal neuromuscular disorder caused by mutations in the DMD gene that result in the absence of dystrophin. Lack of dystrophin results in progressive muscle weakness, degeneration and wasting, leading to premature death due to cardiorespiratory failure (1,2). Currently, there is no cure for DMD, and available treatments are aimed at alleviating symptoms (3). There is great interest in generating therapeutic approaches for the treatment of musculoskeletal-associated pathologies. Research in this area has focused on understanding the molecular and cellular mechanisms regulating skeletal muscle regeneration, to identify and develop new therapeutic approaches to counteract and/or ameliorate the most significant symptoms of this pathology. Obestatin/GPR39 is an autocrine/paracrine system regulating myogenesis and involved in the initiation of skeletal muscle regeneration. This system can modify dystrophic skeletal muscle towards an oxidative phenotype, increasing its functionality (endurance and strength) and reducing muscle pathology, which seems to be associated with an increase in the biosynthesis of proteins such as uthrophin and α7β1-integrins (4).

In this study, we set out to molecularly characterise the obestatin/GPR39 system in the regulation of autophagy as a key tool aimed at reducing muscle damage in DMD. In vitro results showed that the obestatin/GPR39 system activates the main regulatory signalling node of autophagy, ULK1, through the simultaneous activation of the CAMKII-AMPK, and Akt/mTOR axes. The temporal coupling of anabolic and catabolic machineries is required for the restoration and maintenance of protein biosynthesis in DMD.

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# Temporal fluctuations of the HIV-1 protease stability under diverse evolutionary scenarios

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The trade-off from acquiring resistance against the host immune system and antiretroviral therapies while maintaining protein function is crucial for HIV-1 protein drug targets. In particular, resistance mutations allow the virus to escape drug therapies but also could produce viral costs by altering the protein folding stability and activity. Concerning the latter, here we explored the evolution of the HIV-1 protease (PR) folding stability with particular interest in the consequences of relevant resistance mutations. We also evaluated relationships between the PR folding stability and global viral fitness in multiple HIV-1 patients monitored over time. We found that the HIV-1 PR folding stability fluctuated over time and do not correlate with diverse clinical parameters such as viral load and CD4 count. In general, resistance mutations decrease the HIV-1 PR folding stability, although some of them can maintain or increase it. We conclude that the HIV-1 PR presents large structural flexibility to accommodate resistance mutations without constraining the overall virus fitness and evolution.

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# Human breast milk MicroRNAs as potential regulators in development brain.

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Human brain development is a long process that begins in the 3rd gestational week and extends through late adolescence, arguably throughout the lifespan. It ranges from molecular events to environmental inputs, interacting between each other, and eventually defining brain development[1]. If something wrong happens, it could cause future mental disorders[2]. Breast milk (BM) contains complex nutrients that facilitates the maturation of various biological systems in infants. BM also contains microRNAs (miRNAs), in particular in exosomes[3], membranous endocytic vesicles (30–100nm), released by a variety of cell types into the extracellular space, but their putative biological role has not been fully elucidated[4].

Samples from human mature BM and colostrum were collected form the Human Milk Banc of Vigo, and stored at –20°C until exosome extraction. This project was approved by Galician Ethics Committee (2016/559). 36 samples were grouped according to the time of delivering and milk states: mature milk and colostrum at term; premature milk and colostrum; and preterm mature milk. Exosome extraction were done with EX04 Exo-spin$^{TM}$ midi column, optimized by our laboratory. Exosome were detected by WB and TEM. Total RNA was extracted and purified and 260/280 and 260/230 purity ratios were determined. RIN were assessed on a 2100 Bioanalyzer. Libraries were prepared by RealSeq Biosciences and sequenced on a NextSeq 550 v2 High-Output-SR 75 Cycle. The raw fastq files were processed using Cutadapt 2.3 with Python 3.6.8. Reads were aligned to the human dataset in miRBase with Bowtie. Differential expression analysis was performed using the DESeq2 software. miRNA targets, functional Gene Ontology (GO) enrichment analysis and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis were performed with miRPathDB 2.0. Selected GO and KEGG categories had p-value<1e$^{-3}$.

73 miRNAs had significant differential expression after paired group comparison. 70 had at least one match for nervous system pathway (e.g., dopaminergic/glutamatergic synapse, neurotransmitter secretion), biological process (e.g., neuron projection morphogenesis, synaptic vesicle transport,) or cellular component (e.g., postsynaptic membrane, axon, presynpase). Human BM has many miRNAs whose targets may play significant roles in the development and normal function of the new-born nervous system.

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# Comparison of the neurotoxic effects of two doses of microplastics

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Plastic pollution is considered one of the main environmental problems. Multiple studies have documented that microplastics are ingested by various marine organisms, accumulate in specific tissues, and are transported through the food chain to mammals, posing a potential risk to the health of human populations. Experimental data shows that, in fish, these derivatives can cross the blood-brain barrier, reach the brain and cause positive regulation of genes in the CNS, affect the activity of acetylcholinesterase and the dopaminergic system. The main objective of the present work is to evaluate the effects of the administration of microplastics on the biochemical parameters in the levels of acetylcholinesterase (Acetylcholinesterases are enzymes that hydrolyze the neurotransmitter acetylcholine, we measured the amount of enzyme that catalyzes the production of micromoles of thiocholine per minute using the Ellman method) and the antioxidant capacity (measurement of the total non-enzymatic antioxidant capacity of biological samples is indicative of their ability to counteract cell damage induced by oxidative stress) determined by colorimetric methods in different areas of the nervous system (cerebellum, brainstem, cerebral cortex, striatum, and hippocampus). Sprague-Dawley female rats (250-300 g, n = 5/experimental group) were used for the experiments, performing three experimental groups: control and treated whit 5 or 10 mg/kg of polystyrene microplastics (1 µM) for 4 days by systemic administration (via intraperitoneal). The data were statistically evaluated by ANOVA/Dunnet test**.** There was a significant increase in the concentration of antioxidants in the hippocampus of the treated animals with 5mg/kg of microplastic (1.08 ± 0.22 and 1.57 ± 0.36 nmol and 3,31 ± 0,83 / microliter of antioxidant, $p < 0.05$ ) and a significant decrease in the concentration of antioxidants in the cerebellum of the treated animals with 10 mg/kg of microplastic (1.71 ± 0.47, 1.18 ± 0.25 and 0.82 ± 0.11 microliter of antioxidant, $p < 0.05$) In the rest of the areas, no significant differences were obtained. No significant differences were found in acetylcholinesterase levels in different brain areas. With the results obtained, we see that microplastics produce an effect on oxidative stress, but more experiments are needed with other doses of administration and other treatment times.

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# Advances in the synthesis of eupenicinicols C and D based on an intramolecular Diels-Alder reaction

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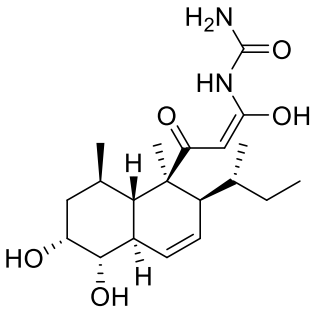
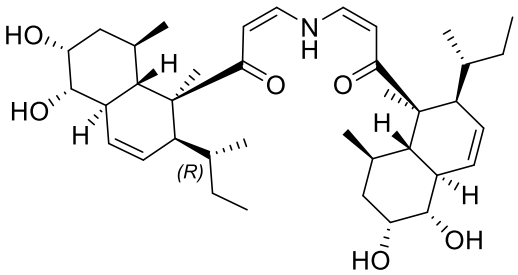
In recent years, the use of small molecules as epigenetic inhibitors has been extended to induce the appearance of new secondary metabolites in microorganisms associated with plants.[1,2] This is the case of the metabolites that are members of the eupenicinicol family, isolated from an endophytic fungus of a Chinese medicinal plant *Xanthium sibiricum*, which are capable of producing decalin-moiety-containing compounds.[3]

Motivated by the interesting biological profiles of these metabolites and our interest in natural products with decalin structure, herein we describe our first results on the stereoselective synthesis of eupenicinicol C and D, to obtain enough of both metabolites to complete the study of their biological profiles.




**eupenicinicol C**




**eupenicinicol D**

***Figure 1**: Structures of eupenicinicol C and eupenicinicol D.*

***Acknowledgment:*** *MINECO (PID2019-107855RB-100) and Xunta de Galicia ((Consolidación GRC ED431 C 2021/45 from DXPCTSUG; ED-431G/02-FEDER "Unha maneira de facer Europa" to CINBIO, a Galician research centre).*

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# Molecular evolution of SARS-CoV-2 in Galicia

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SARS-CoV-2 is a beta-coronavirus responsible for the COVID-19 pandemic. Understanding its molecular evolution is fundamental to identify the mechanisms that modulate its transmision rate, provoke drug resistance or permit vaccine escape. Concerning the pandemic in Galicia (NW Spain), the consortium "EPICOVIGAL", conformed by local hospitals and universities (https://epicovigal.uvigo.es) has sequenced more than 2,000 SARS-CoV-2 genomes from infected individuals in this region. In this study, we analyzed the evolutionary processes acting on these genomes, including recombination, nucleotide substitution and molecular adaptation, with a particular focus on structural and non-structural genes. Overall, we observed a few recombination events and genome-wide purifying selection, but also some positively selected sites that suggest local adaptations along the genome. These results are in agreement with those derived in other regions of the world.

# Extracellular matrix components involved in schizophrenia

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Emerging evidence suggests a critical role of the brain extracellular matrix in the pathogenesis of schizophrenia (SZ), which involves neuronal migration, proliferation and differentiation, regulation of neurodevelopment, neuroplasticity, axon guidance, and neurite outgrowth. The extracellular matrix (ECM) presents specific biochemical and biophysical properties. Its structure is dynamic and varies in response to stimuli and physiological alterations. Thus, a slight alteration can modify the physicochemical state of tissue and alter cell-ECM interactions and cellular phenotypes, which leads to disease development. A progressive neurodegeneration characterized by loss of neurons and ECM composition abnormalities contributes to pathological features of SZ [1].

To mediate an immune response, lymphocytes must be able to interact with and respond to the surrounding extracellular environment. Recent studies have demonstrated that lymphocytes interact with components of the ECM in processes like migration, recognition/activation, and differentiation [2].

In the present study we aim to detect and quantify ECM components in serum and lymphocytes of SZ patients (n = 40) and healthy subjects (n = 40). Peripheral blood mononuclear cells (PBMCs) isolation and serum extraction was performed from blood samples by density gradient centrifugation with Ficoll. Protein quantification is performed through western blot. The proteins selected were LRP1, MMP9, TIMP3, ITGB2, ITGB3, FN1, TUBA and CALR. The previous results show no significant differences between healthy subjects and SZ patients for all the proteins analysed in serum samples. Once the results in lymphocytes are finished, protein quantification will be correlated between the serum and PBMCs to assess potential differences. Although the study is not yet complete, it's great important to evaluate these differences since the brain ECM is essential to several neural functions in the CNS.

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# Evolutionary origin and mechanisms of the pupillary reflex in vertebrates

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The pupillary reflex is present in most vertebrates and allows adapting the amount of light that penetrates the eye and, therefore, that affects the retina, according to the luminosity of each environment. This enables to maximize imaging efficiency, while minimizing retinal damage. This reflex is well studied and defined in some vertebrates such as several mammals, but its evolutionary origins remain unclear. The pupillary reflex is mediated by the Edinger–Westphal nucleus via the ciliary ganglion [1]. Additionally, in some vertebrates a second slower accommodative mechanism exists that is intrinsic to the intraocular muscles located in the iris, mediated by melanopsin present in the fibers of the sphincter pupillae muscle, whose conformation changes with light, causing pupil reduction or dilatation, respectively [2].

Lampreys belong to the oldest group of living vertebrates, diverged about 560 million years ago, but they show a well-developed nervous system with many neuronal mechanisms that have been preserved throughout evolution, including all basic components of the visuo-vestibular control of gaze and all the main types of eye movements [3]. Thus, their use is advantageous due to the simplicity of their circuitry, that facilitates their dissection, and to the knowledge they provide regarding the evolution of vertebrates. The existence of the pupillary reflex has recently been reported in lampreys [4], but its mechanisms have not been determined yet. In the present study, the pupillary reflex mechanisms were analyzed in these animals to clarify the evolutionary origin of this visual function. Our results show that, at least, the intrinsic mechanism of intraocular muscles also occurs in lamprey, what means that pupillary accommodation has been present since the beginning of vertebrates' evolution.

*This work was supported by the grant Proyectos I+D+i PID2020-113646GA-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe", and the Ramón y Cajal grant RYC2018-024053-I funded by MCIN/AEI/10.13039/501100011033 and by "ESF Investing in your Future," Xunta de Galicia (ED431B 2021/04), and CINBIO.*

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# A new HIV-1 Protease Substitution Model based on Functional constrains

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Substitution models of evolution should be as realistic as possible to obtain evolutionary inferences with biological meaning. In this concern, it is known that stability constrained substitution (SCS) models of protein evolution are more realistic than the traditional empirical substitution models. However, there is room for improvement by additionally considering functional constraints in the model. Here, we present the development of a new site-specific SCS model of HIV protease (PR) evolution that accounts for the protein function by quantifying the protease–substrate binding interactions (binding free energy obtained from distance contacts, hydrogen bond network perturbation, disruption of the hydrophobic core and diverse physicochemical interactions). We also present the performance of this new substitution model, that accounts for the protein function, by likelihood-based evaluations of its fitting with real HIV PR data.

*This work was supported by the Spanish Ministry of Economy and Competitiveness [grant numbers RYC-2015-18241 and PID2019-107931GA-I00].*

# Efficient extraction of cancer biomarkers from whole blood using a modular microfluidic platform

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Cancer is a dynamic and heterogeneous disease[1], and metastasis are the main cause of mortality in cancer[2]. Existing clinical monitoring tools, namely tissue biopsy, fail to detect metastatic lesions early and cannot always be performed in a metastatic setting, not to mention the invasiveness of the procedure. The presence of tumor components in patients' blood, and their dissemination is the main mechanism of metastasis formation, their analysis is the ideal approach to monitor disease progression. Liquid biopsy(LB) is a minimally invasive method that provides real-time information about tumor progression, overcoming the limitations of tissue biopsy, allowing early and accurate diagnosis, and personalized therapy[3]. LB can analyze biomarkers like CTCs, EVs and ctDNA. CTCs are tumor cells in peripheral blood with significant prognostic value for MC providing information on tumor characteristics. With origin in necrotic/apoptotic cells, ctDNA appears in plasma and carries tumor-related mutations, relevant to cancer progression and therapy resistance. Also found in plasma, EVs are tumor cell fragments, containing similar proteins and RNA/DNA, responsible of cell-to-cell communication, promoting cancer progression. The analysis of these biomarkers together can gather much more information on cancer progression than when studied individually[3], but extracting all biomarkers from the same blood sample is challenging. Microfluidics presents numerous advantages to handle biological samples[4], making it ideal for clinical practice, enabling high throughput, portability and automation. So, the aim of this project is to develop an innovative and effective modular microfluidic system that is able to isolate all relevant circulating biomarkers from one single blood sample, after microfluidic CTC isolation[5], for comprehensive cancer progression monitoring through LB: 1) Effective separation of plasma from blood cells; 2) cfDNA extraction from plasma; 3) EVs isolation from plasma. Different designs were reformulated and adapted for each module using CAD software, and fabrication was achieved using lithography techniques. The device will be functionalized and the efficiency will be evaluated and optimized in spiking experiments using different protocols.

*This work was supported by the FCT funded scholarship with the reference 2020.05533.BD.*

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# Epigenetic inhibitors as a potential alternative treatment to overcome drug resistance in pancreatic cancer

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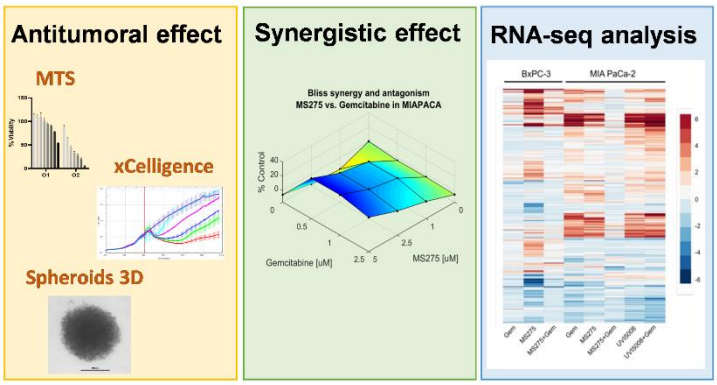
Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive carcinoma with a poor prognosis, and no effective treatment at present. Consequently, there is an urgent need to test new, unconventional, and combined therapeutic drugs to improve the prognosis and survival of PDAC patients.

Reverting the aberrant tumour-induced epigenetic modifications by using epigenetic enzyme inhibitors has recently emerged as an alternative therapeutic strategy on PDAC [1]. For that reason, we conducted systematic cell viability studies with five different epigenetic inhibitors, targeting either HDAC1/4, DNMT3a, EHMT2 or SIRT1 on three different human PDAC cell lines (MIAPaCa-2, BxPC-3 and SKPC-1), alone or in combination with gemcitabine, by using 2 dimension (2D) and 3D cell models. The synergistic effect of these antitumoral drugs with gemcitabine was also tested and the most efficient combinations were characterized by RNA-seq. The cell viability studies showed that three of the epigenetic inhibitors had a good antitumoral effect on the three cell lines. In addition, a high synergistic score with gemcitabine was found for two of them (UVI5008 [2] and MS275 [3]), even in the partially gemcitabine-resistant MIAPaCa-2 cells. Interestingly, the antitumoral effect by the drug combinations was observed at up to 10-fold lower doses than with the individual treatments, reducing the potential associated toxicity, and the RNA-seq analysis revealed some potential biomarkers as synergy candidate genes. In summary, the use of the epigenetic inhibitors UVI5008 and MS275 in combination with gemcitabine offers an alternative treatment for PDAC patients, with an important reduction of the therapeutic dose.




***Funding:*** *government of Spain (ref. BIO2017-84974-R) and Xunta de Galicia (GRC-[ED431C 2020/02]) and Centro singular de investigación de Galicia (ERDF)-[ED431G2019/06].*

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# Development of a microfluidic device for the isolation of ctDNA from the blood of cancer patients

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Breast cancer is one of the most common cancer types and a leading cause of cancer-related mortality in women [1]. The methods currently used in clinic to monitor metastasis are not ideal, as they fail to give information about the tumour subtype.

Through liquid biopsy (LB), which is a minimally invasive method, it is possible to access information about the tumour in real-time, overcoming the limitations of tissue biopsy, and allowing a more precise and early diagnosis and personalized therapy. LB analyses several cancer biomarkers, including ctDNA [2].

Circulating tumor DNA (ctDNA) is fragmented DNA released from tumor cells released upon apoptosis, necrosis and/or autophagy that circulates freely in the patient's blood. ctDNA [3]. Previous ctDNA studies have shown that it represents a valuable biomarker as it represents a real-time source of tumor-related information, relevant to cancer progression and personalize patient treatment. However, current techniques for ctDNA extraction are complicated and not very efficient [4]

Microfluidic is the area of science of handling and controlling fluids and particles in microchannels [5]. It presents multiple advantages to handle biological samples, such as the possibility of portable, disposable, and inexpensive devices, and the offer of integration capability so that the entire range of benchtop laboratory protocols, from sample handling to reaction, separation and detection, can be incorporated and automated on a single chip [6].

The objective of this work is to develop a microfluidic tool to isolate ctDNA from whole blood, which is relevant for early detection of cancer at the point of care, accurate prognosis, and personalized treatment. The device will be functionalized with a positively charged compound, allowing electrostatic interactions with the DNA fragments. To analyze the efficiency of the module, known concentrations of DNA fragments will be spiked and extraction efficiency will be quantified using the Qubit Fluorometer system.

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# An Integrated Microfluidic System for the Manipulation of Circulating Tumor Cells (CTCs)

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In recent years, liquid biopsy has emerged as an attractive non-invasive alternative to tissue biopsy. This approach focuses on the analysis of tumour biomarkers present in body fluids, such as circulating tumour cells (CTCs). [1] [2] Specifically, CTCs have proved to be clinically valuable for tumour prediction, monitoring, and treatment designation.[3]

Therefore, there is an urgency to effectively and reliably detect and isolate CTCs. Moreover, apart from isolation, there is still the need to access the captured CTCs, to carry out further downstream analysis, such as single-cell genomics or functional studies. [4]

In this context, the main objective of this project is to establish the interface between the isolation and recovery of CTCs and the subsequent encapsulation and sorting of CTCs in microdroplets for single-cell analysis. The successful integration of these microfluidic modules may pave the way to obtain a more detailed and in-depth profiling of cancer cells and a better understanding of their role of in each patient.

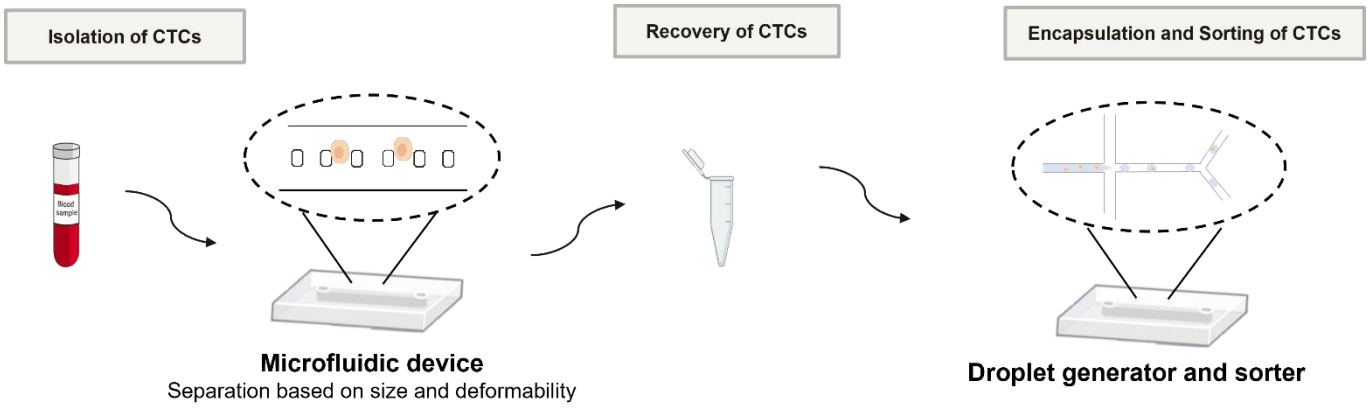


***Figure 1 -*** *Project Overview*

*The Project leading to these results has received funding and support from the H2020 project funded under the program EXCELLENT SCIENCE - Future and Emerging Technologies (FET) BIOCELLPHE (GA* 965018) *and the "La Caixa" Foundation under La Caixa Health program (PROMISE-HR20-00637)*

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# Progress on the Stereocontrolled Synthesis of Staphyloxanthin

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Staphyloxanthin[1] is a natural product, isolated from the *Staphylococcus aureus* bacteria strains, formed by three structural fragments: a glucose core, a branched quiral-C15 fatty acid (of unknown configuration) linked at the primary alcohol and a carotenoid carboxylic acid connected at the anomeric carbon.[2] Taking into account the relevant role of staphyloxanthin in the resistance of the immune system against antibiotics, its synthesis would allow the development of new and more effective antibacterial treatments.

Herein, we report our initial results on the stereocontrolled synthesis of the carotenoid fragment of staphyloxanthin.

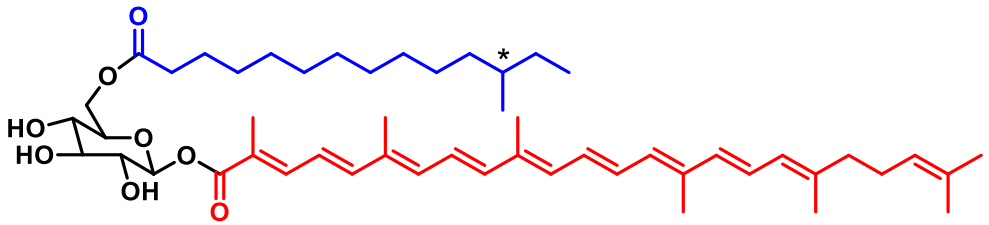


**Figure 1.** Structure of staphyloxanthin.

***Acknowledgment:*** *MINECO (PID2019-107855RB-100) and Xunta de Galicia ((Consolidación GRC ED431 C 2021/45 from DXPCTSUG; ED-431G/02-FEDER "Unha maneira de facer Europa" to CINBIO, a Galician Research Centre).*

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# Towards the Total Synthesis of Ciromicin A

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The family of polyenic macrolactams is a heterogeneous group of secondary metabolites that contain, among other structural features, a cyclic amide group, and a conjugated polyene chain of specific configuration. Examples of natural products belonging to this group are vicenistatin, macrotermycin, auroramycin, silvalactam and ciromicin A.[1] Their biological activities span from antifungal and antibiotic properties for silvalactam and auroramycin, to cytotoxic activity against human leukemia cells in the case of ciromicin A. This natural product was isolated from Nocardiopsis/RW co-culture fermentation, and its structure elucidated after extensive HRMS and NMR analysis.[2]

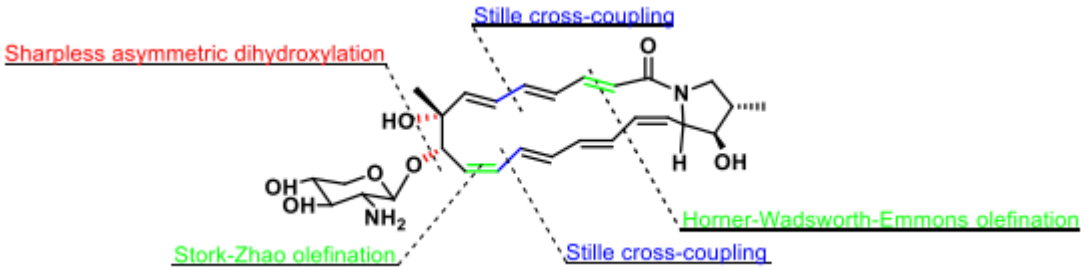


***Figure** 1: Key steps for the Total Synthesis of the proposed structure of ciromicin A.*

In this project, we will explore a synthetic route to the synthesis of ciromicin A using methodologies that allow exquisite control of the geometries of the newly formed $Csp^2$=$Csp^2$ bonds, e.g., Horner-Wadsworth-Emmons and Stork-Zhao olefinations,[4] and the enantioselective formation of the vicinal diol moiety present in the molecule using the Sharpless asymmetric dihydroxylation. Furthermore, palladium-catalyzed cross-coupling reactions[5] will be used to generate new single bonds between $sp^2$ carbons with high stereoselectivity.

*MINECO (PID2019-107855RB-100), Xunta de Galicia ((Consolidación GRC ED431 C 2021/45 from DXPCTSUG; ED-431G/02-FEDER "Unha maneira de facer Europa" to CINBIO, a Galician research centre) are acknowledged for funding.*

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# Autonomous isolation and characterisation of circulating tumour cells from whole blood

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Liquid biopsy has been introduced as a novel strategy in the diagnosis and monitoring of cancer progression. It lies in the isolation and analysis of diverse cancer-derived biomarkers contained in body fluids, including circulating tumour cells (CTCs), following the onset and spreading of cells from the primary tumour. This methodology itself has numerous advantages over the gold standard tissue biopsy: it is minimally invasive, inexpensive, highly sensitive, and can be used for real-time patient monitoring. Importantly, the analysis of this biomarkers can be used to influence patient's treatment, enabling the realisation of personalised medicine [1].

CTCs carry vital information about tumour heterogeneity and play a crucial role during metastasis. Therefore, devising simple yet powerful strategies for their isolation leveraging their biological or physical characteristics is one of the driving forces in the battle against cancer. Although CTC isolation based on the expression of specific proteins on their surface is the most sought strategy, size-based isolation methods employing microfluidic strategies have recently demostrated their enormous potential for unbiased CTC detection [2]. In fact, our group has developed the microfluidic RUBYchip™ which has been validated in the clinic with success, in a variety of cancer types [3, 4]. To enable the implementation of this technology as a diagnostic system, it is crucial to automate the process, minimising user interaction.

In this work, the microfluidic chip has therefore been integrated into an *in-house* developed fully automated sample handling unit, that I sable to perform all tasks from CTC isolation to cell labelling. The automated unit is composed of two injection blocks, and equipped with sensors and electronic actuators, and it is fully controlled by a computer. It accommodates a standard 10 mL vacutainer, acting as the blood reservoir and allows the automatic handling and injection of 12 distinct solutions. The performance of the system has been evaluated by comparing the CTC isolation efficiency against the manual procedure, using spiked samples.

*This work has received funding and support from the "la Caixa" Foundation under CaixaImpulse Grant LCF/TR/CC20/52480003 (CTC-OD).*

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# Multiplexed Optical Fiber Biosensors for Cancer Biomarkers

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Optical fiber technology has been a revolution in the telecommunication field during the last decades. However, the recent advances in nanotechnology and surface engineering have allowed the implementation of this technology in other fields, including its application as sensors and biosensors [1]. These developments motivated the project Bio-FOCS that is taking place between the Medical devices group of INL (Portugal) and the group of Optics, photonics and biophotonics of UPM (Spain). The goal of this project is to take advantage of optical fiber technology to develop a modular multiplexed sensing system for the detection of different cancer biomarkers. This multiplexed detection will take place thanks to the combination of several optical transducers based on different detection principles. The characteristics of optical fibers will allow the engineering of a versatile portable system, and the use of a shared read-out platform and microfluidics will contribute towards the construction of a cost-effective system that will use a reduced amount of reagents and samples. As a proof of concept, this system will be used to monitor the presence and concentration of lactate, thiocyanate, IL-6, oxygen, $CO_2$, and pH. To have a more accurate profile of the samples, the sensor signals will be analyzed with the help of artificial intelligence techniques [2].

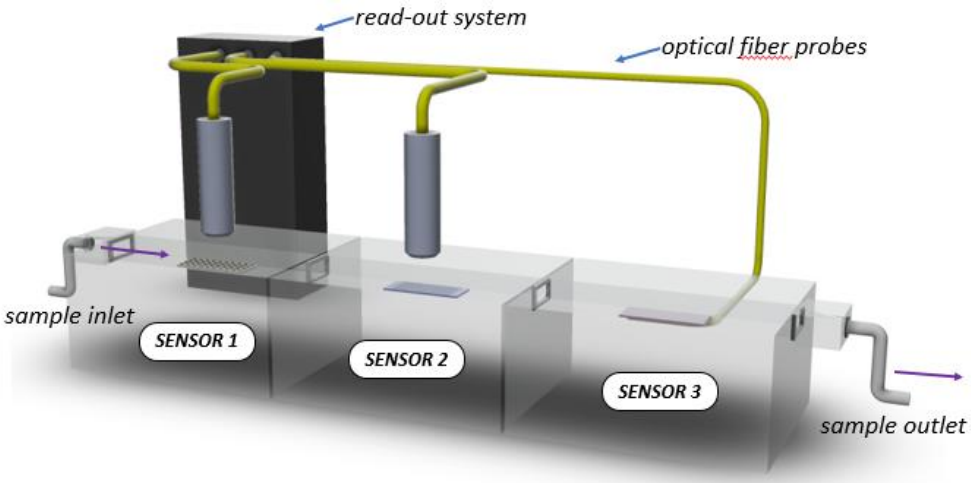


***Figure 1.*** *Concept of the optical platform for biosensing*

*This work is supported by European Union-NextGenerationEU under the grant RD 289/2021.*

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# Involvement of cytoskeleton proteins in cell communication and in Alzheimer's disease

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INTRODUCTION AND OBJECTIVES: The immune system is involved in the functioning of the central nervous system, and with this, one of the main types of cells, lymphocytes, somehow regulate brain function. In addition, from studies with proteomic techniques we know that the proteins of the cytoskeleton of lymphocytes and other cells such as Vinculin, PIP5K1C, Paxillin, Talin, RIAM, β3 integrin and β2 integrin are involved in the pathophysiological mechanisms of several mental disorders such as major depression, bipolar disorder, as well as neurological disorders such as Alzheimer's disease. All of them form a network in the plasma membrane to establish cell communication. Since lymphocytes serve as a comparative study model to investigate what happens in neuronal pathways, we set out to investigate whether there are alterations in these proteins that allow us to know how the cytoskeleton is altered in this disease.

MATERIAL AND METHODS: A comparative study was developed with 20 patients with Alzheimer's disease, 10 patients with major depression and 20 healthy controls, where the levels of vinculin, paxillin, talin, PIP5K1C, RIAM, integrin β3 and integrin β2 in lymphocytes were measured, plasma and cerebrospinal fluid. The patients with Alzheimer's disease were evaluated by the Dementia Unit of the Álvaro Cunqueiro Hospital.

RESULTS: We compared the levels of these proteins between the total number of patients (N = 30) and controls (N = 20) using ANOVA. Vinculin, β3 integrin, β2 integrin, RIAM, paxillin levels in lymphocytes and cerebrospinal fluid were lower in patients with Alzheimer's disease compared to control; however, only with PIP5K1C we detected higher levels with respect to the controls. In plasma, the values of vinculin, β3 integrin, β2 integrin, RIAM and talin are higher in patients with Alzheimer's, quite the opposite of what happens in cases of major depression.

CONCLUSIONS: The levels of cytoskeleton proteins (Vinculin, β3 integrin, β2 integrin, RIAM, PIP5K1C, Paxillin and Talin) are involved in cell communication and are altered in Alzheimer's pathology. This would indicate that there are molecular changes in lymphocytes, as well as in plasma and cerebrospinal fluid that may somehow modulate the pathophysiology of Alzheimer's disease.

# Protective effects of antioxidants on striatal dopamine release induced by organophosphorus pesticides

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**Introduction.** Classically, the toxic effects of organophosphorus (OP) pesticides have been attributed to inhibition of the enzyme acetylcholinesterase (AChE): excessive accumulation of acetylcholine, and overstimulation of its receptors [1]. However, in the acute poisoning, other neurotoxic mechanisms that are not dependent on AChE inhibition may occur. Thus, it has also been shown that these pesticides can generate oxidative stress, one of the main mechanisms of OP neurotoxicity [2, 3]. The purpose of the present work was to evaluate whether antioxidants glutathione (GSH) and trolox prevents the excessive dopamine (DA) release induced by the OP pesticides paraoxon (POX), glufosinate (GLA), and glyphosate (GLY) in conscious and freely moving rats, using cerebral microdialysis technique.

**Methods.** Female Sprague-Dawley adult rats (250-350 g, 4/10 group) were used in the experiments. POX, GLA, GLY, GSH, and trolox were administered directly into the striatum through a microdialysis probe. Levels of DA were measured using HPLC-EC. Statistical analysis was made by means of ANOVA and Student-Newman-Keuls test. Significant differences were as follow: $P<0.05$, $P<0.01$ and $P<0.001$.

**Results.** Infusion of POX (5 mM), GLA (10 mM) or GLY (5 mM) significantly increased the DA release to 1006±106%, 991±142%, and 1164±128%, relative to baseline, respectively. To evaluate if these increased DA release could be related to oxidative stress, we pretreated animals with GHS or trolox before and during administration of OP pesticides. Treatment of animals with 400 µM GSH before and during POX, GLA, or GLY infusion decreased DA release by 65,2%, 61,5% and 100%, when compared to the effect of pesticides alone, respectively. Pretreatment of animals with 1 mM trolox before and during POX infusion had no significant effects on DA levels when compared with the effect of POX alone. On the other hand, infusion of GLA or GLY to animals pretreated with trolox had no significant effects on DA levels, relative to baseline, that is, trolox completely prevented the increase in DA release induced by both pesticides.

**Conclusions:** The antioxidants GSH and trolox were highly effective in preventing the GLY- and GLA-induced increases in DA overflow, supporting the hypothesis that acute exposure to these pesticides and increased DA levels could lead to lipoperoxidation, alteration in total antioxidant capacity, or disturbed total thiol molecules, as the antioxidants significantly decreased the effect of GLY and GLA on DA release. However, only GSH produced a significant decrease in the POX-induced DA release. The high toxicity of this pesticide could explain this lack of effect in our experimental conditions.

***Financial support**: Xunta de Galicia (Nº CI8-GPC 2019, REF ED 431B 2019/33*).

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# Synthetic pathway for the first Gemini-type analog of maxacalcitol

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Calcitriol or 1⟨,25-dihidroxivitamina-$D_3$ is the hormonally active form of vitamin D and its main function is calcium and phosphorus homeostasis in the organism but is has been demonstrated that this molecule also intervenes in other relevant biological processes[1] such as control of cell proliferation, inducement of cell apoptosis and regulation of different biological processes. Due to these non-classical functions, calcitriol could be used to treat numerous illnesses. Nevertheless, its therapeutic application implies serious hypercalcemic effects.

Therefore, analogs of 1⟨,25-dihidroxivitamina-$D_3$ commenced to be studied in order to obtain more specificity for these non-classical functions and reduce the calcemic activity. Among these analogs highlight oxacalcitriol, which has an oxygen atom in 22 position and less calcemic effects than calcitriol[2] , and Gemini-type analogs, which have a double side chain[3] and high selectivity for antiproliferative activity.

We aimed to bring together the advantages of these analogs so we synthesized novel oxacalcitriol Gemini analog **1**. For this purpose, available commercial Inhoffen diol was used to obtain ketone **3**, which is starting material in a synthetic route towards diol **2**, with total stereocontrol over 20 position, which is a key intermediate in the synthesis of Gemini-type analags of maxacalcitol.

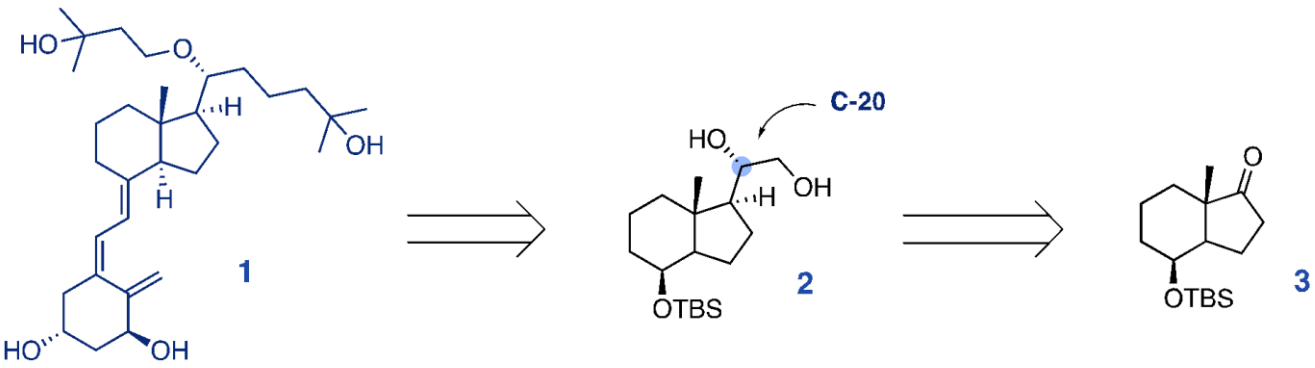


***Figure 1.*** *Retrosynthetic approach to obtain novel oxacalcitriol Gemini analog.*

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# Mutational characterization of the CORO2B gene and its relationship to ciliopathies

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**Background/Objectives**:

The CORO2B gene belongs to the family of actin-binding coronin proteins involved in cellular processes such as cell division, cell migration and movement, vesicle trafficking within the cytosol and phagocytosis These processes are often altered in ciliopathies, one of them is Bardet Biedl syndrome (BBS MIM# 209900), a multisystem disease whose main symptoms are retinal degeneration, obesity, polydactyly, mental retardation, cryptorchidism and defects in renal structure and function. This high genetic heterogeneity cannot fully explain the large inter- and intra-familial phenotypic variability when members of the same family carry the same causal variant(s), suggesting that other mechanisms are involved in the development of the phenotype.

**Methods:**

Exome sequencing of two patients with clinical suspicion of BBS, subsequent validation by Sanger sequencing. Functional analyses were composed of confocal microscopy of the CORO2B subcellular localization and quantification of expression levels. Immunoprecipitation and cofactor proteomics are currently undergoing.

**Results:**

We describe two new mutations in the CORO2B gene placing it as a possible candidate to modulate the BBS phenotype. In the present work we functionally characterize a set of 3 mutations (Ala 129 Val, Leu 194 Gln and Pro 318 Leu), two found by sequencing and one more extracted from VarSome.

**Conclusion:**

We identified novel the mutations Ala 129 Val, Leu 194 Gln and Pro 318 Leu in the CORO2B gene in two BBS patients. Functional analyses indicate that Ala 129 Val and Pro 318 Leu could have a pathogenic character.

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*CINBIO has financial support from Xunta de Galicia and the European Union (European Regional Development Fund - ERDF) (PO FEDER ED431G/02).*
*C.S. is supported by a Ministerio de Universidades FPU predoctoral fellowship (FPU19/00175).*

# Comparison of two nucleic acid enrichment kits for the analysis of the fecal bacteriome

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The microorganisms that inhabit the human body make up the human microbiome, which can be divided in turn into a virome (viruses) and bacteriome (bacteria) component. Changes in these microbial communities, in particular in the bacteriome, are known to be directly related to health and disease [1,2]. Several laboratory kits have been designed for the enrichment and extraction of microbial nucleic acids from human samples. In this study, we evaluated the performance of two of these kits, *AllPrep® PowerViral® DNA/RNA, Qiagen®* and *ZymoBIOMICS™ DNA Mini Kit, Zymo Research®*, for the analysis of the bacteriome in feces samples from five patients. We included two technical replicates per kit and patient,captured the bacterial fraction by targeting the whole 16S rRNA gene by PCR, and sequenced the resulting libraries on a MiniSeq Illumina platform (2x150 bp). Using the taxonomic classification system *kraken2* [3] with the SILVA [4] database, we found that more than 95% of the sequencing reads mapped to bacteria. In general, we obtained a similar bacterial composition across patients. The predominant phylum was *Firmicutes*, followed by *Bacteroidota* and *Proteobacteria*. Furthermore, we estimated the alpha and beta diversity with the R package *vegan* [5] and observed no significant differences between the two kits tested. Our results indicate that both kits performed similarly and are suitable for the study of the bacteriome in feces samples. The capacity of the kits for the enrichment of the virome fraction should be tested in the future for the joint analysis of the bacteriome and the virome in human samples.

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# Nanozymes with improved catalytic properties

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Natural enzymes are extremely efficient at catalyzing a huge variety of reactions with high substrate specify, activities and yields under mild reaction conditions. As a result, there has been a significant interest in using them for diverse applications. However, problems as low operational stability, sensitivity to environmental conditions, difficulties in recovery and recycling limit their applications. To overcome these limitations great efforts have been made to explore efficient mimetic materials of these natural enzymes. Although nowadays it is well established that nanomaterials mimicking natural enzymes, nanozymes, posse several distinct advantages over natural enzymes as well as other reported artificial enzymes, they still face several limitations.

Aptamers are sequence-specific nucleic acids exhibiting selective binding properties towards low-molecular-weight substrates and macromolecules to which a catalytic unit can be tether. In this work the covalent linkage of aptamer binding sites to nanozymes, “aptananozymes”, is introduced as a versatile method to improve the selectivity and catalytic activity of nanozymes by concentrating the reaction substrates at the catalytic nanozyme core, thereby emulating the binding and catalytic active-site functions of native enzymes.

The concept was exemplified with the synthesis of Prussian blue (PB) nanozymes, functionalized with the L-hydroxy arginine binding aptamer for the $H_2O_2$-mediated oxidation of N-hydroxy-L-arginine to L-citrulline. The aptananozymes reveal enhanced catalytic activities as compared to the separated catalyst and respective aptamer constituents.

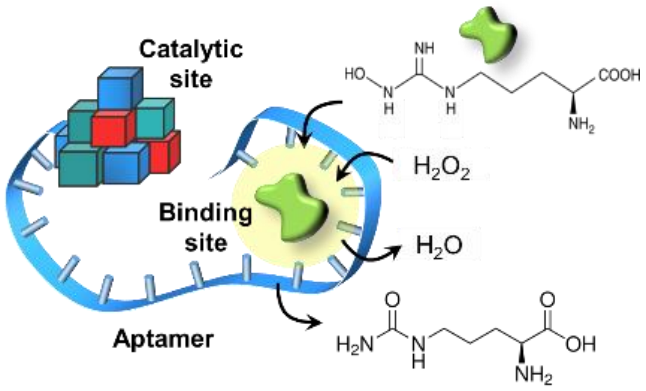


***Figure.*** *Schematic model of the catalytic “aptananozymes”*

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# Real time Monitoring of Liver Inflammation in a Mouse Model: A Showcase of Luminescent Nanothermometers at Deep Organs

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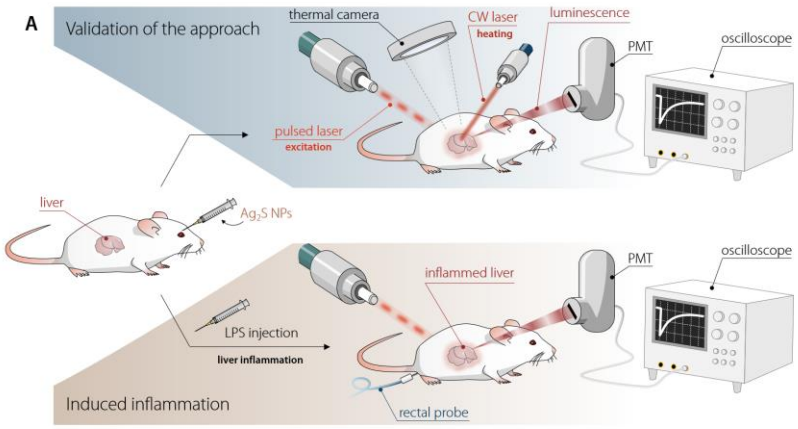
Temperature of tissues and organs is one of the first parameters affected by physiological and pathological processes, such as metabolic activity, acute trauma, or infection-induced inflammation. Therefore, the onset and development of these processes can be detected by monitoring deviations from basal temperature. To accomplish this, minimally invasive, reliable, and accurate measurement of the absolute temperature of internal organs is required. Luminescence nanothermometry is the ideal technology for meeting these requirements. Although this technique has lately undergone remarkable developments, its reliability is being questioned due to spectral distortions caused by biological tissues. In this work, how the use of bright $Ag_2S$ nanoparticles featuring temperature-dependent fluorescence lifetime enables reliable and accurate measurement of the absolute temperature of the liver in mice subjected to lipopolysaccharide-induced inflammation is demonstrated. Beyond the remarkable thermal sensitivity (≈ 3% °C–1 around 37 °C) and thermal resolution obtained (smaller than 0.3 °C), the results included in this work set a blueprint for the development of new diagnostic procedures based on the use of intracorporeal temperature as a physiological indicator.




***Real-time thermal monitoring of liver during inflammation.*** *Schematic representation of the experimental procedure designed to monitor liver temperature during LPS-induced inflammation [1].*

*Funding from Comunidad de Madrid through TALENTO grant ref. 2019-T1/IND-14014 is acknowledged*

**References:**

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# Feasibility of $ZrO_2$-based nanofluids for geothermal applications through their thermophysical profile

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Turbulent forced convection processes are the most widespread in industrial applications, being water, glycols, or mixtures of both commonly used as working fluids. The characterization of the thermophysical profile of these fluids is fundamental to design the equipment and the processes in which they are involved. Achieving improvements on this field has been one of the main objectives of the past decades to reduce energy consumption. Likewise, since the low thermal conductivity of these conventional fluids hinders the heat transfer mechanism, there is still a need for improvement in this matter. Nanofluids, dispersions of solid nanoparticles in a base fluid, have been proposed as a potential solution to overcome this limitation [1]. Thermal conductivity is an influencing parameter in the heat transfer performance of a fluid because it accounts its ability to dissipate or absorb energy when a temperature gradient disturbs it from the thermal equilibrium. However, the dynamic viscosity should be also considered from a practical approach because high penalties in this property can compromise the pumping power and therefore the efficiency of the system. Propylene glycol is a non-toxic compound, which makes it an attractive choice for antifreezing protection, and it is widely used in geothermal applications [2]. In this study, the thermal conductivity and dynamic viscosity of five mass concentrations of zirconium oxide nanoparticles (0.25, 0.50, 0.75, 1.0, and 5.0 wt%) dispersed in a binary mixture of propylene glycol and water at 10:90 vol% are analysed at different temperatures. It was found that the nanoparticle loading increased both thermal conductivity and dynamic viscosity of up to 2.8% and 33%, respectively.

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# Highly-sensitive nanostar SERS substrate for cancer metabolite detection

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Surface-enhanced Raman scattering (SERS) is a powerful and sensitive technique for the detection of chemical fingerprint signals of molecules, becoming more popular in several areas - medical diagnostics, environmental, and food safety[1]. Typical bottom-up approaches use chemical approaches to produce metallic nanoparticles in a wide variety of morphologies like nanoflowers and nanostars that are deposited on a substrate. The sharp edges by these nanostructures account for the creation of hot spots where the local signal enhancement takes place[2]. However, the control of these hot spots is not trivial and it is a bottleneck the SERS sensing developments are facing in order to be effectively translated.

Alternatively, top-down approaches such as micro- and nanofabricated substrates have been developed to produce surfaces with metallic nanostructures array. These allow for a precise control of interparticle distance, a more predictable enhancement factor, high signal homogeneity along the substrate and a unique focal plane for analysis. In this work, we have developed a high density SERS array with different diameters of Au nanostars (50 – 650 nm) at different interparticle distances or pitches (200, 300, 500, 700 nm). Each of the nanostars holds multiple hot spots at their tips leading to very intense SERS signals. The SERS efficiency of these substrates were tested by using a standard Raman Reporter (RaR), the 1-Naphthalenethiol (1NAT). For a further proof-of-concept we used this novel substrate to detect trypotophan, a metabolite expressed by some tumours [3]. Generally, the metabolome is involved in cellular physiology maintenance, such as mediating cellular communications, activating cell receptors or feeding energy in biochemical cycles. These novel SERS substrates may shed light on the metabolic processes involved in cancer development, cell to cell communication or microenvironment conditions favouring cancer replication.

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# Chiral Perovskites for Optoelectronics

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Lead halide perovskites are an emerging class of semiconductors that are in expansion due to their inherent optoelectronic properties which are particularly suitable in device performance. However, its potential is limited due to its centrosymmetric structure, which can be detrimental to some of the required properties. Accordingly, recent studies have shown the possibility of breaking this spatial parity by incorporating chiral organic ligands into the perovskite nanocrystal framework.[1, 2] The induction of chirality, allows them to exhibit additional remarkable properties such as circular dichroism, circularly polarized photoluminescence, nonlinear optical effects, ferroelectricity and spintronics. However, despite numerous studies, their dissymmetry factors are still far from the high values obtained for classical QDs, and the mechanism of chirality induction is still under debate. Therefore, it is essential to explore new strategies to optimize the degree of chirality and its optoelectronic properties.

In this context, we have focused on the development of new approaches to obtain chiral perovskite nanocrystals of controlled composition and shape. Specifically, we have focused on 2D perovskites due to their higher surface-to-volume ratio, which favors a higher density of chiral ligands leading to an increase in the degree of chirality. For this purpose, we have employed chiral organic ligands that we have incorporated into perovskites either by direct synthesis or by post-synthetic surface passivation, applying these methodologies to thin films as well as to colloidal nanocrystals. In addition, we have modulated its band-gap by varying the halogen mixing ratio, resulting in a corresponding signal shift. The comprehensive study of the behavior of chiral perovskite nanocrystals enables us to define the influencing factors for optimizing the degree of chirality taking a step forward for its use in device performance.

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# Theoretical Study of Bimetallic Plasmonic Nanostars and their Efficiency as Photocatalysts

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The study and design of metal NPs with high HE generation efficiencies are of great interest in applications such as photocatalysis [1], and NPs with strong electric fields near to their surface are interesting for biosensing applications [2]. Nanostars (NS) show a combination of remarkable properties for such goals: quasi-spherical symmetry providing a consistent response in colloidal suspensions, narrow tips with strong hot-spots and strong tunability through different geometrical parameters. Silver NPs generally offer stronger HE efficiencies [3], but gold NPs offer us an easier way to reach near-IR resonances. In this work, then, we aim to perform a theoretical study of the optical properties, the HE generation efficiency and the field enhancement (FE) displayed by gold nanostars (AuNS), silver nanostars (AgNS), AuNS with a layer of silver (AuNS@Ag) and AgNS with a layer of gold (AgNS@Au), exploring the possibility of synergistic effects in the combination of the two metals. For the AuNS case, we will analyse the dependence of all the magnitudes of interest with the length and the width of the spikes; and for the coated stars, we will discuss the effect of the thickness of the external metal layer in their optical properties. Aiding this study, we had access to experimental characterization of bimetallic nanostars previously synthesized in the group. Then, the creation of the geometries under consideration was guided by data from Scanning Transmission Electron Microscopy (STEM) images and absorbance spectra of these systems.

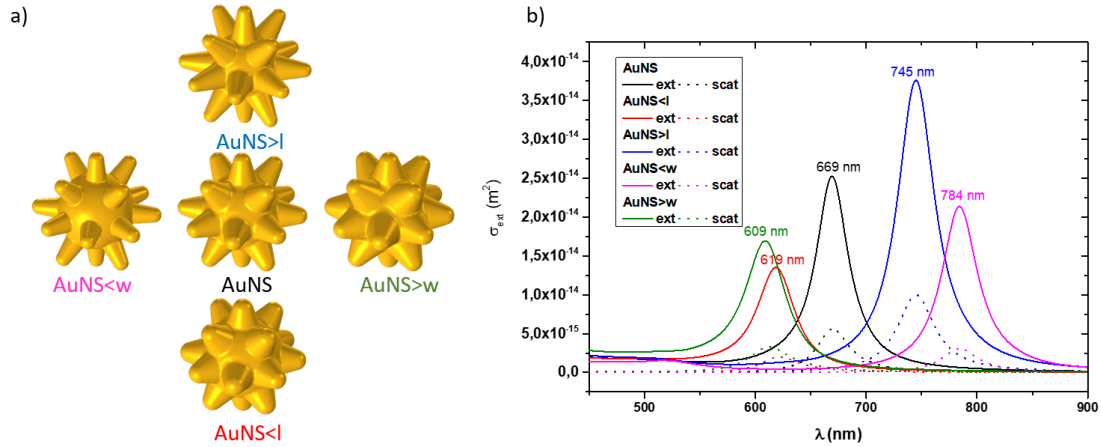


***Figure** a) Visual comparison of the five models with spikes with different aspect ratio. b) Extinction (solid lines) and scattering (dashed lines) spectra of the five different gold nanostars.*

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# A microfluidic tool for rare cell proliferation, expansion and study

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Fighting cancer metastasis efficiently remains a challenge in the oncology field. Metastasis involves a set of complex events, with malignant cells from primary tumors invading the surrounding tissues and entering blood circulation to finally spread to distant organs. These circulating tumor cells (CTCs) are thus directly responsible of cancer progression, making them a crucial target for early detection and relapse prevention, as well as primary candidates to unravel the mechanisms of metastasis, invasiveness, resistance, and ultimately aiding therapeutic selection and personalized treatment [1]. Critically, CTC isolation, recovery and proliferation for their incorporation in functional studies still remain highly challenging and ambitious tasks. Taking advantage of microfluidics, the RUBYchip™ has shown to provide fast and efficient isolation of viable CTCs based on their size and deformability, and has recently demonstrated its clinical validity on a wide range of metastatic cancers [3-4]. Moreover, previous results showed that isolated cells can be recovered from the isolation device and encapsulated in microdroplets [5]. Such droplets allow for the generation of self-contained, isolated microenvironments where encapsulated cells have the capability to grow, and downstream analysis can be performed without risking cell viability. This work aims to develop a platform to expand the small sample of patient-derived CTCs inside microdroplets with assistance of physiologically relevant hydrogels. For that purpose, two key challenges must be addressed. First, studies on a variety of culturing conditions and hydrogels must be performed to arrive at the optimal conditions for CTC growth and generation of small clumps, mimicking metastasis formation. Second, given the low number of CTCs collected per patient, a sorting system will be developed to specifically encapsulate and collect only the droplets containing the valuable CTCs. Preliminary results using passive droplet sorting methods and GFP-labelled cancer cells as a model, demonstrate cell viability during the first 72 h post-encapsulation with several hydrogels. Further, *in vitro* testing of samples with small cell concentrations and highly supplemented hydrogels/mediums seem to promote cell proliferation and clump formation, potentially prolonguing CTC viability within microdroplets. With the microfluidic tools and rare cell proliferation methods developed within this work, viable CTC-derived clumps will be generated and, posteriorly, transferred into organ-on-a-chip systems to study CTC intra- and extravasation. We expect this new platform to reveal relevant information about the underlying mechanisms of metastasis.

*This work was supported by the PROMISE project funded through the Caixa Health Research program from La Caixa Foundation (HR20-00637).*

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# PVDF-gold SERS substrates for the analysis of cell lysates

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Understanding and investigating the differences between health and disease states at the cellular level are very significant towards reaching a proper early diagnosis and personalised treatment. Surface-enhanced Raman scattering (SERS) spectroscopy is considered one of the emergent techniques used to efficiently analyze cell lysate samples, due to its high sensitivity and specificity.[1] In fact, the analysis of cell lysates can provide us with unique matrices that reflect specific biochemical information which can be related to their health/disease state. Here, we developed low-cost paper-based SERS substrates to analyze different cell samples towards discriminating healthy from tumour tissue. The substrates were fabricated by functionalizing polyvinylidene difluoride (PVDF) membranes with polydopamine first, followed by in-situ reduction of gold. Polydopamine was chosen as a biocompatible green reducing agent to deposit gold nanostructures onto the micropores of PVDF membranes.[2] We optimized the SERS performance based on the concentration of polydopamine and its polymerization time while keeping the gold concentration constant. Then, we further optimized the SERS performance by varying the concentration of gold precursor and its reaction time. The optimized substrates were applied for label-free discrimination between the cellular lysates from cancerous and non-cancerous cell lines, such as human colon adenocarcinoma (SW480) and peripheral blood mononuclear cells (PBMCs), respectively. SERS can open new avenues to extract insightful biochemical information at the cellular level that can lead to the development of promising diagnostic, therapeutic, and theranostic tools.

*The authors wish to acknowledge the "Innovative Microfluidic Platform for Analysis of myeloid Leukemia blasts" IMPAct-L project (030782) co-funded by FCT and the ERDF through COMPETE2020 as well as BIOCELLPHE project (H2020-FETOPEN-2018-2020, grant agreement ID: 965018). A. T acknowledges the FCT studentship SFRH/BD/148091/2019.*

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# Perspective studies on heat generation of magnetic nanocrystals

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Heat generation by magnetic nanoparticles while exposed to an alternating magnetic field (AMF) has become an exhaustively studied topic in nowadays research, given the application in many fields as biomedicine or catalysis.[1][2] Alas, the experimental determination of the heating capabilities is yet not fully standardised, thus causing difficulties in the understanding and making the comparison of the obtained experimental data in different labs a rather challenging task.[3] Herein, we detail some practical insights from calorimetric measurements of $Fe_3O_4$/γ-$Fe_2O_3$, $CoFe_2O_4$, $MnFe_2O_4$ nanocrystals, previously characterised by X-ray diffraction, transmission electron microscopy, and Raman spectroscopy, aiming to correlate the obtained SAR values with the magnetic properties registered, to comprehend the heat transfer performance of the different examples.[4]

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# Development of a microfluidic device for the isolation of tumour-derived extracellular vesicles

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Breast Cancer (BC) is the most common type of cancer diagnosed in the European Union, with an estimated incidence of 13,3 % in 2020[1]. Diagnosis of relapse by clinical imaging has low sensitivity, resulting in a decreased prognosis for patients with metastatic disease[1]. Thus, understanding BC pathophysiology is key to improving clinical diagnosis and therapeutic decisions; however, new biomarker detection strategies are currently lacking. Extracellular vesicles (EVs) are cell-secreted, lipid-bound particles that can be found in circulation in the blood and other body fluids, being responsible for cell-to-cell communication. EVs also contain valuable genetic cargo, proteins, and other biomarkers that can be clinically relevant. However, isolation of EVs remains challenging, relying mostly on centrifugation strategies which are in general time consuming and inherently imply low yield. Recently, the ExoGAG reagent has been introduced as a method to streamline EV enrichment allowing their isolation from liquid biopsies (i.e. patient-derived blood samples) and obtaining, as a result, a purified EV population to study their content[2]. Microfluidic devices have shown recently promising EV isolation results[3], collecting EVs with low contaminations, while manipulating fluids at high flow rates. Specifically, micromixers can be used to enhance the generation of EV-ExoGAG complexes, increasing the efficiency of EV collection. The goal of this project is to create a microsystem that can isolate EVs from liquid biopsies for early BC diagnosis. Preliminary results (**Figure 1**) on the optimization of micromixers for different flow rates, geometrical designs, and hydrodynamic mechanics have shown that an efficient mixing of ExoGAG and EVs should be possible, opening the door for enhanced EV enrichment from patient-derived samples.

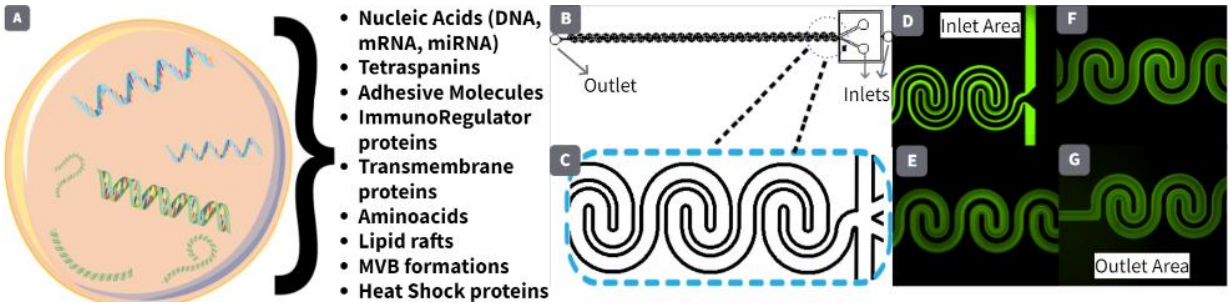


**Figure 1** - Micromixer for EV analysis. **A** – EV content. **B** – Layout of the micromixer with zoomed-in on the entrance microchannels (**C**). Mixing assay using fluorescein along with the device (**D**, **E**, **F**, and **G**).

*This work has received funding and support from the "la Caixa" Foundation under CaixaImpulse Grant LCF/TR/CC20/52480003 (CTC-OD).*

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# UV-A activated platinum nanoparticles synthetized with natural photosensitizer riboflavin to inhibit cancer cell proliferation

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The search for alternative treatments for difficult cancer types is still of primary importance since the number of yearly cancer deaths still reaches the 10 million [1]. Phototherapies, such as photodynamic therapy (PDT), have a greater efficacy and fewer side-effects. Its therapeutic efficacy derives from the ability of a photosensitizing molecule (PS) to produce cytotoxic singlet oxygen and/or reactive oxygen species upon activation by light irradiation, inducing cell death. Its clinical application has encountered some limitations, such as low PS bioavailability or inefficient light penetration in the tissues. These limitations could be overcome by formulating novel PDT agents consisting in a natural PS conjugated to a metal nanoparticle [2]. Their use improves the bioavailability and enhances the therapeutic efficacy by synergy with other cell death mechanisms. The excellent X-ray attenuation capacity of noble metal nanoparticles could also allow the use of this radiation (XPDT) to treat deep-seated and metastatic tumors. Here, natural PS riboflavin was selected to prepare platinum nanoparticles (RF@Pt). RF@Pt were completely characterized by different techniques, and they were studied *in vitro* in A549 adenocarcinomic human alveolar basal epithelial cells to assess their biocompatibility, toxicity, and therapeutical efficacy. Confocal microscopy confirmed cytoplasmic internalization of RF@Pt by A549 cells. The cell counts showed a 2-fold decrease of viable cells during RF@Pt treatment (300µg/mL) after irradiation (UV-A 375 nm). Combined treatment (RF@Pt + irradiation) also caused oxidative stress, evidenced by a decrease in intracellular GSH level and by an increased expression of HO-1.

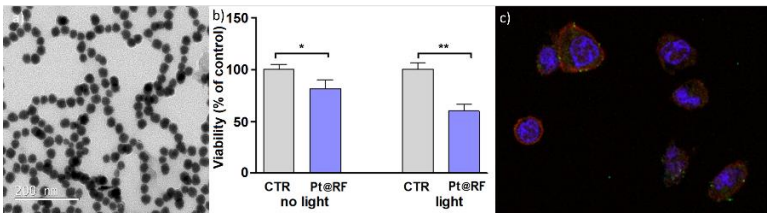


***Figure**. a) TEM image of RF@Pt b) cell viability of A459 after Pt@RF treatment and after Pt@RF + UV-A activation c) confocal microscopy image of RF@Pt internalized in A549 cytoplasm.*

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# Engineered metalloproteins for bioorthogonal transformations

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The bioorthogonal approach aims to develop *in vivo* reactions that do not disturb the cellular metabolism and viability, whilst exploiting the intracellular environment to obtain valuable products for biological applications. Although recent advances in chemical methods for transition metal-powered bioorthogonal catalysis have been quite impressive [1], some limitations persist, mainly in the extent of integration with biolo-gical systems, catalyst deactivation and metal-related toxicity. To solve this, proteins appear as the ideal candidates to host metal centres in biological settings overcoming the aforementioned drawbacks, just like nature does with the metalloenzymes that catalyse most metabolic reactions. In this regard, miniproteins have already been used to staple the metal and allow its internalisation [2]. Following this thread, we have turned our attention to exploit the possibilities offered by modular engineered proteins, in particular the *Consensus TetratricoPeptide Repeat* (CTPR) motif, already established as a robust scaffold for biomolecular engineering [3], to promote bioorthogonal catalytic transformations. A variety of discrete-ion- and na-nocluster-bearing CTPR-metal conjugates have been prepared, and their activities screened using fluorogenic substrates, with the *in cellulo* reactions monitored by confocal fluorescence microscopy. These novel metal-protein hybrids provide yet another platform for bioorthogonal reactions inside the cell, in what constitutes another advance towards the goal of manipulating the living cell at the chemical level with complete precision and control.

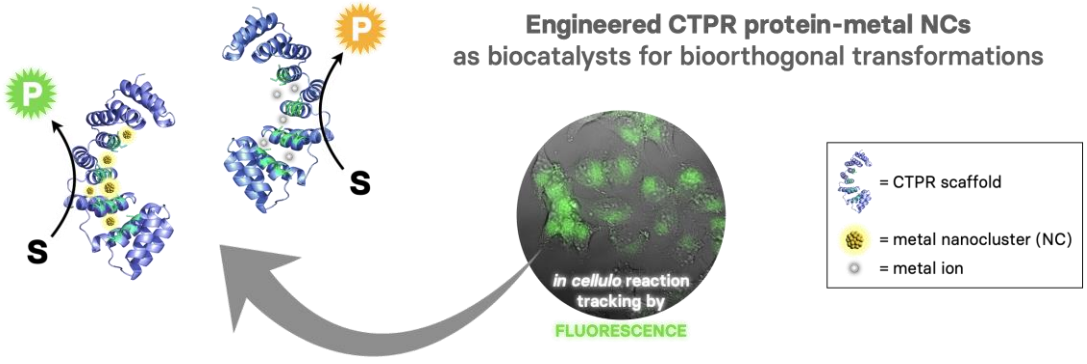


***Figure*** *Graphical summary of the work presented here. S = substrate, P = fluorescent product.*

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# Generation of hot electrons in plasmonic nanoparticles with complex shapes

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The generation of hot electrons in plasmonic nanoparticles is an intrinsic response to light, which strongly depends on the nanoparticle shape, material, and excitation wavelength. In our study, we present a hybrid formalism that incorporates classical and quantum components, which allows us to describe the generation of hot electrons (HEs) in nanoparticles with complex shapes, as well as their energy efficiencies. Although we focused on gold, our approach is suitable for any plasmonic material. Among the geometries we studied (nanospheres, nanorods and nanostars), the nanostars are the most efficient, with an internal energy efficiency of approximately 25%, owing to multiple factors, including the presence of hot spots. This formalism is a convenient tool to design nanoparticles with efficient properties for applications in photochemistry and photodetection.

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# Detection of $TiO_2$ NPs in Seawater through Plasmonic-based Filter System

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Plasmonic gold nanostructures have attracted great interest in recent years due to their optical properties. Particularly, their ability to improve the photocatalytic efficiencies of large bandgap semiconductors such as titanium dioxide nanoparticles ($TiO_2$ NPs) under visible-NIR light irradiation has encouraged their use as photosensitizers. As known, gold nanostars (Au NSts) may significantly enhance the photoactivity of semiconductors under solar light irradiation by means of the enhanced electromagnetic field intensity on their spikes[1]. Herein, synthesized Au NSts were homogeneously deposited on titanate nanowires (Ti NWs), and $TiO_2$ NPs in seawater were collected through the obtained membrane-like structure. The minimum amounts of filtrated $TiO_2$ NPs that could be detected in seawater were investigated through their enhanced photocatalytic activities. Additionally, the fast electron-hole recombination rates between Au NSts and $TiO_2$ NPs were prevented by coating the surface of Au NSts with an insulating silica shell.

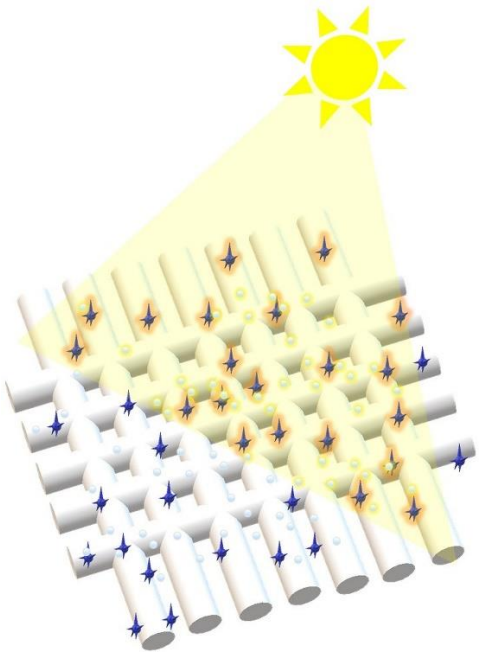

*Figure 2. Schematic presentation of plasmonic filter system under solar light irradiation*

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# Biomimetic nanoparticle assemblies on substrates inspired on the photosynthetic cells of *Cystoseira Tamariscifolia*

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The current climate change crisis required an urgent expansion of the use of renewable energies. Solar energy is becoming increasingly popular across Europe, but to be completely energetically self-sufficient, we need to find new strategies to improve the efficiency of solar cell devices. In this work, we have inspired in the photosynthetic cells of the algae *Cystoseira Tamariscifolia*, which contains photonic crystals formed by close-packed lipid nanospheres surrounded by the chloroplasts.[1] Their function has been suggested to be the optimization of light absorbed modulating the energy collection as a function of the illumination conditions.[1] In this work, inspired by C. Tamariscifolia organelles, we developed photonic crystals composed of core-shell nanoparticles, where silica nanoparticles will play the role of the lipid spheres and J-aggregates are the biomimetic photosynthetic chromophores.

The first step was to prepare colloidal dispersions of these core-shell $SiO_2$-J-aggregate nanoparticles. Commercial silica nanoparticles with a diameter of 245 nm were selected to form a photonic crystal with a bandgap centered at the absorption of the J-aggregate (1,1′-disulfobutyl-3,3′-diethyl-5,5′,6,6′-tetrachlorobenzimidazolyl-carbo-cyanine, TDBC, 590 nm). The nanoparticles were characterized by Transmission Electron Microscopy, Dynamic Light Scattering, Zeta potential and UV-vis absorption spectroscopy. The second step was the preparation of photonic crystals using the so-called vertical method, introducing vertically a glass substrate inside a core-shell $SiO_2$-J-aggregate colloidal dispersion at 60 ºC. The slow evaporation of the solvent induced the nanoparticles deposition on the glass surface.

The photonic crystals were characterised by Scanning Electron Microscopy (SEM), UV-vis absorption spectroscopy and angular reflectometry. The optical response of the biomimetic photonic crystals were analized as a function of: i) the nanoparticle order and spatial distribution, ii) the molecule loading per nanoparticle and, iii) the surface charge of nanoparticles and substrates. For comparison purposes, all the results were compared with thin films constituted of nanoparticles randomly distributed prepared using Layer-by-Layer method.

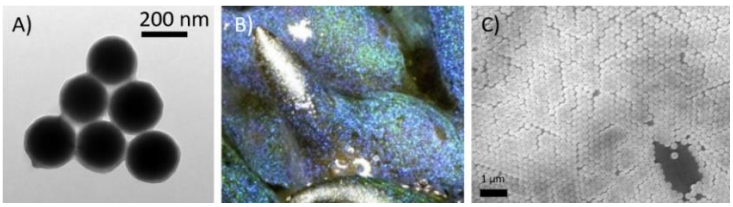


***Figure.*** *A) Seven-layered silica nanoparticles functionalized with PDDA and TDBC characterized by TEM. B) Image of the specimen C. Tamariscifolia with two different colors C) Photonic crystals formed by 245 nm silica nanoparticles deposited on a piranha activated glass substrate*

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# Plasmonic@Metal-Organic Framework (MOF) Yolk-Shell SERS Substrates for Enrichment of Analytes in Aqueous Media

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Surface-enhanced Raman spectroscopy (SERS) has emerged as an ultrasensitive analytical tool with multidisciplinary applications. In recent years, metal-organic frameworks (MOFs) have emerged as an intriguing material to combine with plasmonic nanostructures for designing high-performance hybrid substrates for SERS applications in a wide range of fields, including gas sensing, environmental analysis, and biomedicine. The physicochemical properties of MOFs are highly tunable, which make them excellent candidates for analytical applications. In particular, zeolitic imidazolate frameworks (ZIFs) are a class of MOFs with extraordinary thermal and chemical stability, which can be used in aqueous media, a factor which is particularly relevant for sensing applications. Herein, we report the simple and efficient fabrication of gold nanostars (NS) individually encapsulated into a hollow ZIF-8 shell using ZIF-67 as the sacrificial framework to yield homogenous yolk-shell particles stable in aqueous media. The SERS performances of NS@ZIF-8 yolk-shell substrates were tested using multiple probes, demonstrating higher sensitivity and adsorption capacity of them as compared to their analogous core-shell architectures.

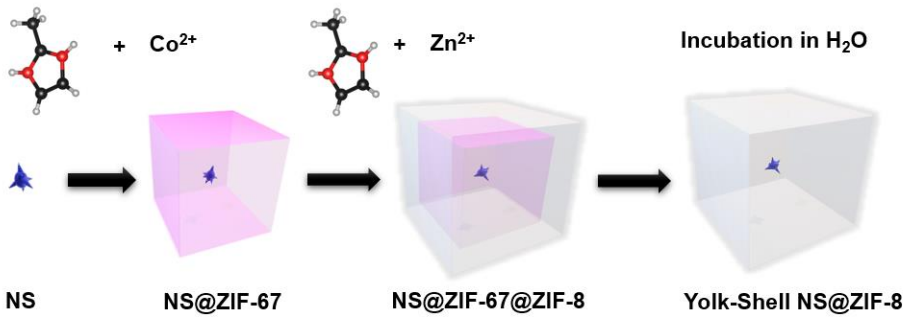


***Figure*** *Preparation of NS@ZIF-8 hybrid yolk-shell SERS substrate*

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# Expression and biochemical characterization of a xylanase obtained by sequence-based metagenomics of a soil sample from Rio Caldo (Lobios, Ourense)

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Today's consumers, concerned about the relationship between nutrition and health, are increasingly demanding functional foods, which combine nutrition and health promotion. Among these foods, prebiotics and probiotics are known to exert beneficial effects due to their interaction with the gut microbiota. While probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host, prebiotics are dietary substances (mainly polysaccharides and non-starch oligosaccharides) that feed certain groups of gut-inhabiting microorganisms by favouring the growth of beneficial bacteria over harmful ones. Since prebiotics are chemical substances, their incorporation into foods offers technological advantages over probiotics since they do not need to be protected from degradation during digestion. Among the best-known common prebiotics are fructans (FOS), inulin, galactooligosaccharides (GOS), lactulose, and breast milk oligosaccharides [1]. Xylooligosaccharides (XOS), products generated by chemical or enzymatic hydrolysis of arabinoxylans present in lignocellulosic biomass, are of increasing interest for their prebiotic effects [2]. The structural characteristics of the XOS, such as their degree of polymerization, branching, linkages, or presence of certain functional groups, determine the microorganisms that can use them as substrates [3]. In this context, the search for new xylanases capable of generating different mixtures of oligomers with different degrees of polymerization and substitution, with potentially different beneficial effects, is of interest.

In the present work, sequence-based metagenomics has been used to isolate a xylanase from soil samples from Río Caldo (with an upwelling temperature between 55 and 75°C) in Lobios, Ourense. Xylanase has been heterologously expressed in yeast and biochemically characterized. It presents an optimum temperature and pH of 70°C and 7 respectively and maintains more than 87% of its activity after incubating one hour at 70º C.

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# Could the extraction technologies have an effect in the results of cell inhibition using seaweeds as raw material?

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Algae are classified according to their majority pigments in green, red and brown, but the composition regarding other fraction is also very different. The main polymer for green is ulvan, for red is carrageenan or agar and for brown is fucoidan. Besides, these biopolymers have different features and properties, but their biological activities are attractive from a point of view of applications. Location, solar light or seawater conditions (temperature or salinity) have influence in the composition of the seaweeds. Also, the extraction technology used to recover these bioactive compounds have an effect in the polymers obtained.

This work was focused on evaluating the cell growth inhibition on a lung tumoral cell line, caused by the oligomers from brown and red seaweeds obtained by ecofriendly technologies, as ultrasound assisted extraction (UAE) and subcritical water extraction (SWE).

The raw material used were: *Sargassum muticum* (brown seaweed), and *Mastocarpus stellatus* (red seaweed). The extraction technologies were UAE, at room temperature and 1:20 ratio solid:liquid (w/w), and SWE during heating up to 170 °C and 1:30 ratio solid:liquid (w/w) for *S. muticum*, and in the case of *M. stellatus*, UAE conditions was room temperature and 1:50 solid:liquid ratio (w/w) and for SWE the temperatures explored were from 70 to 190 °C. In both cases, after extraction solid liquid separation was accomplished and precipitation of alginate with calcium chloride was also performed for the brown seaweed samples. Adenocarcinomic human lung cells (A549) were exposed to the fucoidan and carrageenan oligomers obtained, and the cell inhibition was measured by MTT assay.

The results obtained for the brown seaweed oligomers, produced by SWE (170 °C), have shown cell inhibition around 46% (500 μg/mL) [1], whereas the extracts obtained by UAE exhibited a value of cell inhibition around 25-30%, at the same extract concentration (500 μg/mL) [2]. *M. stellatus* carrageenan obtained by UAE was also explored, showing a growth inhibition higher than 91% for A549 (at 30 μg/mL) [3], and SWE carrageenans showed $IC_{50}$ 0.50 mg/mL and the aqueous extracts without this compounds exhibited 0.41 mg/mL.

The extraction technology influences the composition and structure of fucoidans and carrageenan oligomers, therefore affecting their antiproliferative action.

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# Extraction optimization of fucoxanthin from *Undaria pinnatifida* and evaluation of antioxidant and neuroprotective properties

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Macroalgae are a potential source for functional ingredients. One of them is fucoxanthin (Fx), a common pigment found mostly in brown algae that has gathered the attention of researchers in the last few years because of its biological properties. *Undaria pinnatifida* is one of the most common algae in the Atlantic Ocean, and it is known for its high concentration in Fx [1]. The extraction optimization of Fx was conducted using a heat-assisted extraction of *U. pinnatifida* at a solid-liquid ratio of 30 g/L. Three independent variables were tested: time (*t*, 3 min to 7 days), temperature (*T*, 5 to 65ºC) and concentration of acetone in water as the extraction solvent (*S*, 50 to 100%, v/v). To perform this experimental design a total of 198 independent data points were needed. The responses generated in the experimental design were analyzed with theoretical models for the three main variables (*t*, *T* and *S*) and assembled in a multivariable form to understand the behavior of the extraction process in a much simple form. The predictions made by the models were fitted to the experimental data and statistically confirmed. These results were used in the prediction and optimization of the optimal points. The responses assessed to select the optimal extraction conditions of Fx were: ($Y_1$) the Fx content analyzed by HPLC-DAD; ($Y_2$) the extraction yield and ($Y_3$) the purity of Fx in the obtained extract. All in all, the extraction results obtained are higher than the previous values reported in the literature. Furthermore, some biological properties of the obtained extract were tested. The antioxidant response was evaluated by DPPH, ABTS and Crocin colorimetric assays and the neuroprotective activity was studied by Ellman's colorimetric method. The results reported in this study could be of interest for food and/or pharmaceutical industries, as it describes Fx extraction behavior, which could help to reduce costs and improve the process' efficiency, maximizing the extraction yields and reducing the production costs associated with energy and solvent consumption.

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# Ulvan, a bioactive marine biopolymer

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Green seaweed from the Ulva genus can be attractive alternatives in several fields, from food supplements to biomedical applications for its health benefits due to the presence of biologically active compounds [1]. Ulvan is the main cell wall polysaccharide of *Ulva spp.* green seaweed, which is highly hydrophilic, semi-crystalline in nature and corresponds to around 10-40% dry weight of the macroalga biomass [2]. The bioactivities of ulvan depend mainly on its molecular weight, monosaccharide composition or the sulfate and glyoxylate content, which also drive their rheological features [3]. The features and bioactivities of ulvans can be modulated by the extraction conditions [4]. A remarkable ulvan characteristic is its ability to develop thermo-reversible gels in the presence of calcium ions at a pH around 7.5 [5]. The main objective of this research work was the assessment of the influence of a hydrothermal treatment on the features of the recovered ulvans.

*Ulva spp*. green seaweed used as raw material was gently supplied by Portomuíños S.L. (A Coruña, Spain). Hydrothermal extractions using water as solvent were performed on a Microwave reactor Monowave 450 (Anton Paar, Austria). A wide range of thermal conditions over the range from 120 to 200 ºC was tried to recover high valuable fractions. Seaweed samples with a solid:liquid ratio 1:30 (w/w) located in the vials within the microwave device were subjected to a speed rotation of 800 rpm and power of 850 W. After treatment, both solid and liquid phases were vacuum filtrated and the corresponding ulvan precipitated with ethanol from the liquid phase. The structure and rheology of the recovered biopolymeric phase was carefully analyzed.

Results showed a relevant impact of the microwave operation conditions on the extraction yield, sulfate content, molecular weight distribution and mechanical properties of the precipitated ulvans, when compared with those obtained under acid conventional procedure. The lowest tested temperatures promoted the ulvan extraction yields and provided ulvans with higher average molecular weights in addition to enhanced viscoelastic gelling profiles opening up a range of potential bioactive features. The highest sulfate content was identified at intermediate temperatures. These achievements point out the dramatical relevance of selecting adequately the processing conditions of the macroalgae.

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# Vitamin D against cancer: New Gemini analogues

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Calcitriol, the hormonally active form of vitamin $D_3$ has antiproliferative properties and could be used as a therapeutic agent. However, the necessary therapeutic doses involve hypercalcemia risk.[1] Research focuses on the rational design of new calcitriol analogues with higher selectivity. Among the many new calcitriol analogues, worth mentioning those in which the C-21 methyl group was extended to form a second side-chain giving rise to new class of derivatives, known as Gemini and which proved to display less calcemic toxicity.

**UVB1** is a Gemini type analogue recently synthesized in our research group. We showed that this analogue inhibits colorectal carcinoma progression and lacks hypercalcemic activity and toxicity effects in *in vivo* assays.[2]

The first synthesis of this compound was based on a serendipitous result. However, we could not reproduce the same transformation, thus we have developed a new pathway to obtain it from a building block **2** previously synthetized in our laboratory.[3,4]

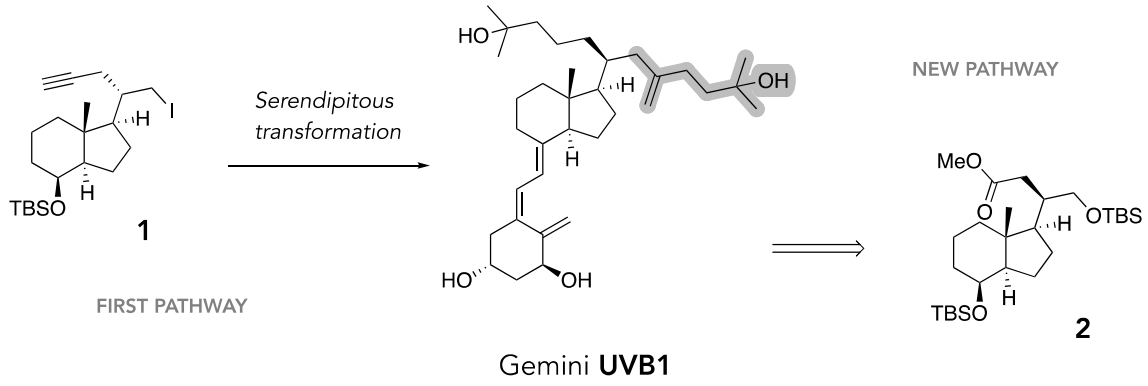


***Figure** Synthesis of UVB1*

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# A novel thermostable GOS-producing β-galactosidase from As Burgas hot spring (Ourense) obtained through functional metagenomics

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β-galactosidases (EC.3.2.1.23) are key enzymes for the hydrolysis of lactose into glucose and galactose. Some β-galactosidases also catalyze transgalactosylation reactions in which the galactosyl moieties are transferred to different acceptor molecules, leading to the production of galacto-oligosaccharides (GOS), non-digestible carbohydrates able to induce the growth of beneficial bifidobacteria. These prebiotics can help in the prevention of colorectal cancer,[1] activation of the immune system,[2] and the enhancement of intestinal mineral absorption,[3] and thus they are frequently added to infant milk formulas, dairy products, and pet food, among others. The use of higher temperatures in the industry can improve the solubility of the substrates, increase the initial productivity of the enzyme and prevent microbial contamination. Therefore, thermostable β-galactosidases have become very interesting for industrial applications. In this study, functional metagenomics has been used for bioprospecting of thermostable β-galactosidases from As Burgas hot spring water. As a result, a novel thermostable β-galactosidase able to produce up to 48% (W/W) of GOS from a solution of 40% (W/V) of lactose at 70ºC has been found and characterized.

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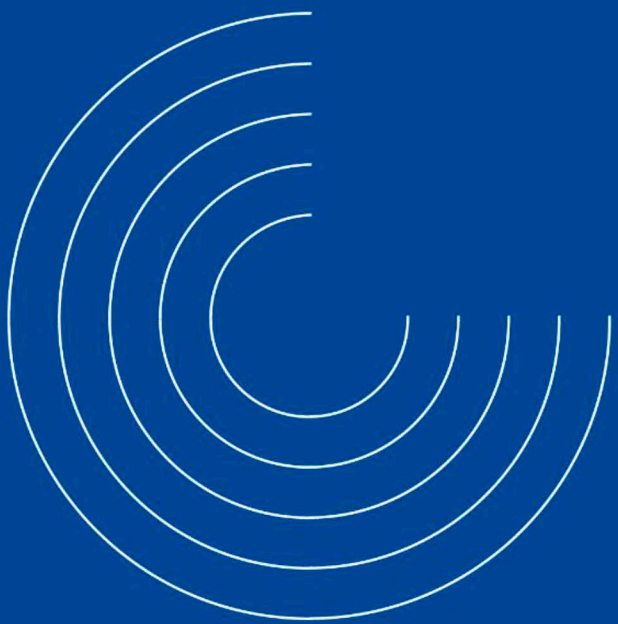


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