V EDITION

PhD Day

Annual Workshop of Young Researchers of CIC biomaGUNE

Book of Abstracts

CIC biomaGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

16th November

Auditorium of the Scientific and Technological Park

About

The "**PhD day of CICbiomaGUNE**" is a yearly meeting open to all researchers, but with a special emphasis on the participation of **PhD students**. The event is envisaged as a Symposium, where PhD students will present their latest results within a relaxed environment and participate in lively scientific debates. First year PhD students will have the opportunity to introduce their research with a poster and the contributions by PhD students of second and following years, will be in the form of short oral communications (5 minutes), encouraging them to focus on presenting the most important results of their research concisely. The best oral communications and posters will receive prizes. The best contributions of the students during the discussions will be also awarded. The students will decide these prizes themselves, and the organization will provide a link to vote after the event.

Ultimately, PhD Day pretends to be a platform for young researchers within the institute to engage in scientific discussion, share ideas and start new collaborations. Furthermore, the event should help to improve the social and working environment at CICbiomaGUNE, which is of vital importance for the scientific development and wellbeing of researchers at the center.

Please remember that *all the content of the event is strictly confidential*. *Only the organizing committee and CICbiomaGUNE will be allowed to record or take pictures, which will not contain sensible information*. This graphic material might be used for promotion purposes in the future. **More information about the data protection policy for the event, and how to withdraw your consent can be found at the end of this booklet**.

Poster session-PhD day 2022

Invited alumni

The “**PhD day of CICbiomaGUNE 2023**” will count with the participation of two alumni as invited speakers, Dr. Alejandro Criado and Dr. Richard Murray.

Dr. Alejandro Criado

Dr Alejandro Criado received his Ph.D. in Organic Chemistry (2013) at the University of Santiago de Compostela (Spain). From 2013 to 2020, he conducted postdoctoral research at the University of Trieste and CICbiomaGUNE research center. In 2021, he started his independent research career as Distinguished Researcher at CICA – Interdisciplinary Center of Chemistry and Biology, Universidade da Coruña, co-leading NanoSelf group. Currently, he is a Ramon y Cajal Assistant Professor at Chemistry Department at Universidade da Coruña. His research interests focus on the new methods for modifying low-dimensional materials to tailor their properties, along with the development of graphene-based sensors.

Dr. Richard Murray

Richard Murray obtained his BSc. (Experimental Physics) and his Ph.D. (Physical Chemistry) at the University of Galway, in Ireland. Following postdoctoral training at CIC BiomaGUNE, Spain, he moved to Berlin for a Marie Cure postdoctoral fellowship. He worked as an associate editor for Wiley's materials science journals, including Advanced Materials, from 2017 to 2019. After spending time working in OA book publishing and project management, Richard re-joined Wiley in 2022. Currently he works as an associate editor on Advanced Functional Materials, Advanced Science, Advanced Optical Materials, Laser & Photonics Reviews, and the recently launched fully Open Access Journal, Advanced Physics Research. Richard is based in San Sebastián.

Thursday, 16th November 2023

Auditorium of the Scientific and Technological Park

9:30-9:45	***Welcome*** ***PhD program in CICbiomaGUNE, Ander Abarrategi***	
9:45-10:15	***Invited Alumni: Alejandro Criado*** ***"Ramón y Cajal" Assistant Professor (Universidade da Coruña)***	
	Session: Synthetic Bioengineering ***Chair: Silvia Collavini***	
10:15-10:20	**Bahaa Doau** *Engineering CNT-based Scaffolds for Neural Tissue Regeneration: Factors Effecting Neuronal Proliferation Within a Synthetic Matrix*	O-1
10:20-10:25	**Nerea Pascual** *3D neuronal monitoring platforms for electrochemical sensing of neurotransmitters*	O-2
10:25-10:30	**Garazi Larrañaga-Jaurrieta** *Scaffolds that mimic the bi-phasic character of the native cartilage for the regeneration of osteoarthritic joints*	O-3
10:30-10:35	**Paula Piñeiro** *Monitoring cell dynamics via 3D SERS imaging using biocompatible SERS tags*	O-4
10:35-10:40	**Maria Regato-Herbella** *Multi-Responsive Hydrogels for Biomedical Applications*	O-5
10:40-10:45	**Paula Vázquez-Aristizabal** *Development of customized bioinks for 3D printed dynamic cancer models*	O-6
10:45-11:20	**Global discussion**	
11:20-11:50	**Coffee break**	

Session: Biofunctional Materials *Chair: Laura Confalonieri*		
11:50-11:55	**Daniel Andrés-Sanz** *Surpassing Substrate–Enzyme Competition by Compartmentalization*	O-7
11:55-12:00	**Ainhoa Maiz-Iginitz** *Scalable enzymatic synthesis of enantiopure β-hydroxy esters and their subsequent polymerization*	O-8
12:00-12:05	**Laura Armero Hernández** *Determination of glycan profiles on biomarkers by Surface-Enhanced Raman Scattering*	O-9
12:05-12:10	**Sergio F. Castillo Pacheco** *Chemoenzymatic Oxidation of Diols Catalyzed by Co-Immobilized Flavins and Dehydrogenases*	O-10
12:10-12:15	***Neda Khatami*** *Valorization of Biological Waste from Insect-based Food Industry: Assessment of Chitin and Chitosan Potential*	O-11
12:15-12:45	**Global discussion**	
12:45-14:30	**Poster Session with Lunch**	

14:30-15:00	***Invited Alumni:*** ***Richard Murray (Wiley Associate Editor)***	

	Session Nanotechnology ***Chair: Gail Anne Vinnacombe-Willson***	
15:00-15:05	**Marita Wagner** *Combined Surface-Enhancement of Infrared and Raman Signals with Gold Antennas*	O-12
15:05-15:10	**Laura Perez-Chirinos** *Tuning the dimensionality of supramolecular materials through the design of peptide-protein co-assemblies*	O-13
15:10-15:15	**Kyle Van Gordon** *Mechanistic investigation of growth on gold nanorods synthesized with chiral inducers*	O-14
15:15-15:20	**Lei Chen** *Impact of the interlayer distance between graphene and MoS2 on Raman Enhancement*	O-15
15:20-15:45	**Global discussion**	

Session Molecular Imaging ***Chair: Mamina Bhol***		
15:45-15:50	**Gabriela Guedes** *Protein-stabilized nanomaterials as MRI contrast agents: one size does not fit all, but versatility does*	O-16
15:50-15:55	**Laura Fernández-Méndez** *Exploring lung surfactant nanoparticles for pulmonary fibrosis*	O-17
15:55-16:00	**Marina Piñol-Cancer** *Evaluating PEG coating of antifibrotic nanoparticles for pulmonary delivery*	O-18
16:00-16:05	**Michele Cesco** *Low dimensional carbon materials as novel multifunctional platforms for biological applications and medical imaging*	O-19
16:05-16:30	**Global discussion**	
16:30	**Starting of Get-together party and Prizes**	

Posters

Oral communications

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Engineering CNT-based Scaffolds for Neural Tissue Regeneration: Factors Effecting Neuronal Proliferation Within a Synthetic Matrix

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Neural regeneration is a complex process marked by astrogliosis and inflammatory cell infiltration into the lesion site, resulting in axonal shortening and substantial demyelination. (1) As a result, efforts are dedicated to impeding the development of glial scars or neuromas. However, currently approved FDA drugs for nerve tissue regeneration, are unable to effectively reverse neural tissue damage on their own. (2) To this end, tissue engineering is a good alternative that uses 3D scaffolds to guide and support axonal growth, more specifically, hydrogels having properties similar to native tissues are promising candidates.

Herein, we present novel hydrogels infused with multiwalled carbon nanotubes (MWCNT) proved to improve synapsis activity and signalling, (3) culminating in patentable biomaterials for nerve tissue regeneration. The hydrogels were synthesized and characterized to study the morphological, chemical, mechanical, and electrical properties including rheology analysis, studying the effect of young's modulus (YM), and the CNT effect on cell growth. They exhibit a network of interconnected fibres engulfing MWCNT having an intrinsic pore size distribution >10 um with the ability to precisely control the pore sizes allowing cell penetration. Scaffolds were then optimized for *in-vitro* studies with SH-SY5Y cells showing no cytotoxicity and favouring growth on the CNT scaffolds. Cells also favor scaffolds with similar YM to that of the native tissues regardless of CNT concentration. Finally, they were studied *in-vivo* on mouse models simulating both crush injury and total excision injury. Preliminary *in vivo* studies showed better wound healing with CNT scaffolds compared to control. all mice presented minimal signs of distress and all scaffolds were found to be biocompatible and non-toxic. CNT implants after 3 weeks showed positive nerve regeneration signs.

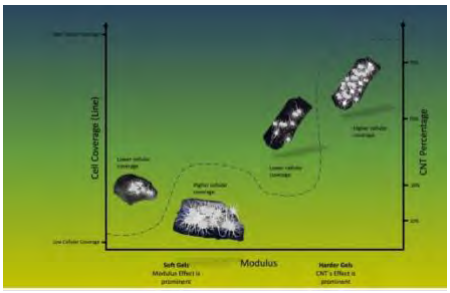

Figure 1. Schematic presentation of various factors effecting cellular growth within a synthetic matrix.

Keywords: Conductive Scaffolds, Hydrogels, Neuronal Tissue Regeneration, MWCNT, Young's Modulus.

References

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3D neuronal monitoring platforms for electrochemical sensing of neurotransmitters

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At the base of several neurological diseases (e.g., Alzheimer's disease, Parkinson's disease) there are neurotransmitters dysfunctions. Thus, determination of neurotransmission dynamics is a compelling phenotype to judge drug-induced neuroprotection. [1] This project aims to develop an electrochemical biosensor of 3D hybrid hydrogel based on extracellular matrix and functionalized carbon nanotubes (f-CNTs) that quantifies neurotransmitters concentration in 3D neuronal cell cultures (Figure 1).

As first step, this study presents a comprehensive investigation into the development of neuronal cultures with distinct neurotransmission phenotypes. Models were developed from induced pluripotent stem cells (iPSC) derived neurons [2] resulting in mature dopaminergic, cholinergic, and glutamatergic neurons with observable morphological traits and functional properties. Characterization included immunochemistry, multi-electrode array recordings, gene expression analysis (rt-PCR), and calcium imaging, demonstrating the maturation and phenotypic characteristics of these neurons. Additionally, the impact of carbon nanotubes (CNTs) on neuronal differentiation was examined, revealing improved cell adhesion and gene expression in the presence of CNTs.

In parallel, an electrochemical sensor platform was designed using Indium-Tin-Oxide (ITO)-coated coverslips with f-CNTs serving as working electrode. The CNTs were functionalized with gold nanoclusters (AuNCs) or cobalt phthalocyanine (CoPC) for dopamine and hydrogen peroxide detection, respectively. The CNT-Au sensor's [3] electrochemical performance was characterized by differential pulse voltammetry, demonstrating a noticeable limit of detection (LOD) of 150nM and a sensitivity of 3,98E-04 A cm^{-2} μM^{-1}. Overall, this study lays the foundation for the development of a 3D neuronal culture monitoring platform coupled to the iPSC derived neurons with potential applications in neuroscience research and drug discovery.

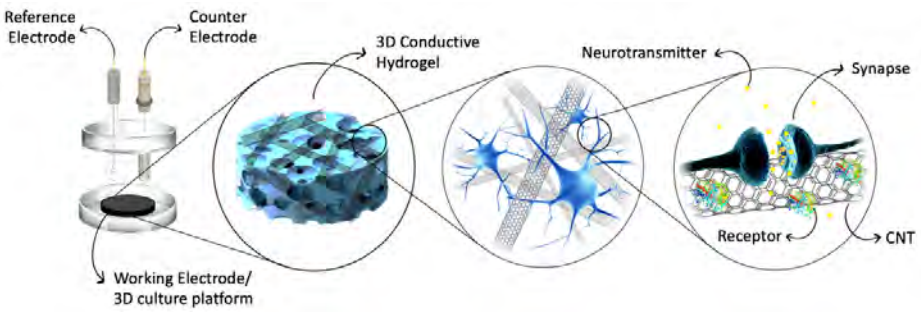

Figure 1. Design of 3D neural monitoring platform for neural network growth and synaptic activity monitoring.

Keywords: neuron, electrochemical biosensor, neurotransmitter, functionalized carbon nanotubes, limit of detection.

References

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Scaffolds that mimic the bi-phasic character of the native cartilage for the regeneration of osteoarthritic joints

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In the native articular cartilage microenvironment, chondrocytes are constantly subjected to dynamic physical stimuli that maintains tissue homeostasis. They produce extra cellular matrix (ECM) components such as collagens (type II mainly, 50-75%), proteoglycans (10-30%) and other type of proteins1. While collagen offers a large resistance in tension, proteoglycans are the responsible of the viscoelastic response under compression due to the negative charge they confer to the ECM allowing it to entrap a large amount of interstitial fluid. In pathologic states (e.g. osteoarthritis), this ECM is degenerated and the negative charge becomes unbalanced, losing the chondroprotective properties and resulting on an overloaded chondrocytes that further degenerate the matrix.

Low-Intensity Pulsed Ultrasound Stimulation (LIPUS) has been used to generate acoustic (pressure) waves that create bubbles that collapse with cells, inducing a stimulus that can modulate cell response2. This mechanical stimulation promotes the expression of type II collagen, type X collagen, aggrecan and TGF-β, appearing as a great strategy to regenerate cartilage. However, current strategies make use of extrinsic forces to stimulate cartilage formation overlooking the physic-chemical properties of the degenerated cartilage, resulting in an excessive load-transfer to chondrocytes and the consequent hypertrophy and degeneration.

Here, interpenetrated networks (IPNs) with different compositions were created using methacrylated gelatin (GelMA), to mimic the collagen, and alginate functionalized with tyramine (Alg-tyr) to mimic glycosaminoglycans and to introduce a negative charge in the model. Within the matrix chondrocytes where encapsulated and stimulated under different conditions to identify the ultrasound parameters that enhance tissue formation.

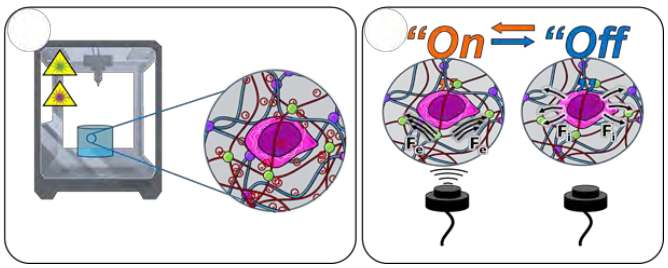

Figure 1. Fabrication of IPNs by 3D-Bioprinting crosslinked with two different wavelengths (left) and the following LIPUS stimulation.

Keywords: Osteoarthritis, IPN, 3D-Bioprinting, LIPUS

References

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Monitoring cell dynamics via 3D SERS imaging using biocompatible SERS tags

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Cell-cell communication is a critical process for the maintenance of biological functions of complex biological systems, mediated by the secretion of extracellular vesicles, mainly exosomes, which transport and deliver relevant biomolecules from a donor to a recipient cell. This process is known to play an important role in various diseases including cancer, and especially in metastatic processes.[1] In order to better understand the role of exosomes in tumor cell dynamics and metastasis, we employed surface-enhanced Raman scattering (SERS) imaging [2] using antibody-functionalized SERS-tags (AB-tags). These nanoparticles are composed of polymer-encapsulated gold nanostars (AuNSs) encoded with various hydrophobic thiolated Raman reporters.[3] By selectively tagging certain cell populations using different Raman active molecules-containing AB-tags, in-situ monitoring of cellular communication can be studied. AB-tag probes with various coatings based on different biomolecules were used to separately label human fibroblasts (HDF) and breast cancer cells (MCF7), and 3D SERS imaging was performed.[4] Multivariate analysis of SERS maps revealed variations in the spatial distribution of SERS signals based on the conjugated antibody employed. The next objective is to monitor SERS tag exchange between various cell populations using AB-tags that can recognize extracellular vesicles by cell encapsulation inside microdroplets employing microfluidics. This will enable us to explore cellular pathways that contribute to disease progression.

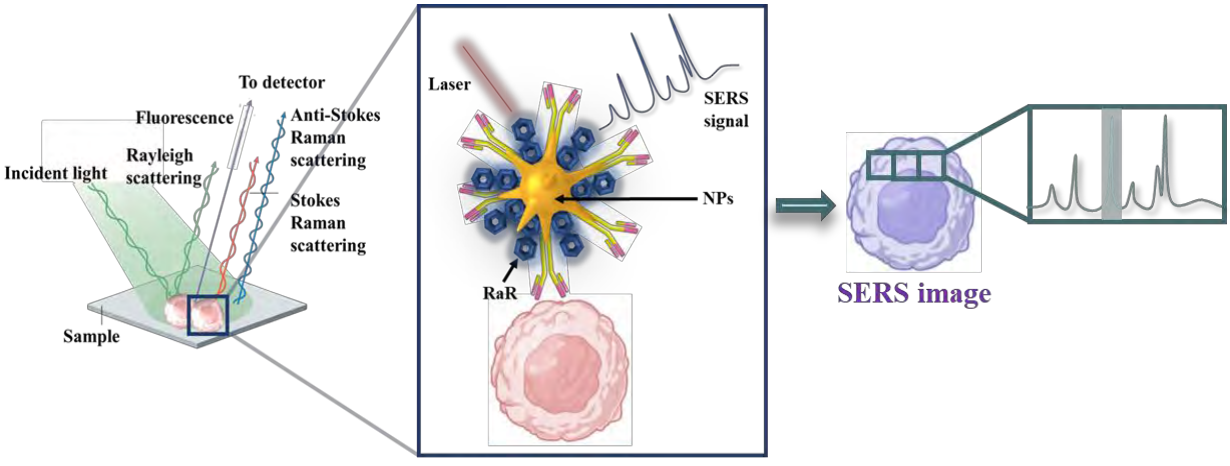

Figure 1: Schematic representation of the SERS imaging of cells labelled with SERS-encoded AuNSs.

Keywords: SERS imaging, SERS tags, cellular communication, extracellular vesicles, antibody targeting

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Multi-Responsive Hydrogels for Biomedical Applications

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Hydrogels are hydrophilic polymeric structures, which retain large amounts of water in a three-dimensional network. In addition to having a high absorption capacity, hydrogels are usually characterized by having high stability, and the capacity to respond to external stimuli while being easy to prepare[1.] Hydrogels can be prepared with a wide variety of monomers, either charged or capable of interacting with water through hydrogen bonding. When immersed in water hydrogels swell, increasing their volume while maintaining their structural integrity[2] (*Figure 1.*). Some of these monomers used to synthesize the hydrogels, have the capacity to respond to stimuli changing the physical and chemical properties and, with it, the hydrophilicity of the structure. There are numerous examples of hydrogels made from polymers responsive to changes in their environment. Responsive hydrogels can be classified according to the type of stimuli to which they respond. Hydrogels may respond to physical stimuli such as temperature, light or electrical stimulation; to chemical stimuli such as pH, redox changes, ionic strength or specific ions and type; and to biological stimuli such as the presence of sugars, enzymes, proteins, and other biologically relevant molecules[3]. Due to the biocompatibility, swelling control and 3D manageable structure, the multi-responsive hydrogels were widely study in some fields, taking special attention in the biomedical field as drug delivery carriers[4].

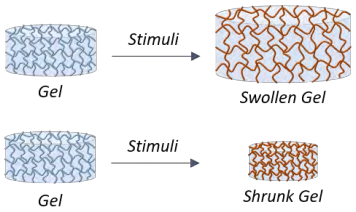

Figure 1. Scheme of multi-responsive hydrogels

Keywords: Multi-Responsive, Hydrogels, temperature, pH, ROS.

References

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Development of customized bioinks for 3D printed dynamic cancer models

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There is an unmet need for more complex and monitorable cancer models. The development of solid tumor micromodels based on printed decellularized extracellular matrices (dECMs) offers a step in this direction, with the biomolecule-rich matrix of dECM inks allowing cell growth in a three-dimensional (3D) and natural environment [1]. By pre-labelling cells with plasmonic nanoparticles (NPs) exhibiting stable and high intensity surface enhanced Raman scattering (SERS) signals, cell movement can be imaged *in situ* using Near-Infrared (NIR) light sources which show limited cytotoxicity and good penetration within biological materials [2]. Hence, the combination of dECM scaffolds and cancer cells labelled with SERS-encoded NPs, will allow the monitoring of cells in a more realistic 3D microenvironment, offering the possibility of studying a living system for longer times than other conventional techniques.
Porcine skin and breast tissue have been effectively decellularized and turned into printable dECM inks that exhibit suitable rheological and biocompatible properties. Additionally, complex 3D cellular models have been developed for both melanoma and breast cancer modelling. By incorporating NPs into the formulations and/or into the spheroids, the cells within the microtissues can be imaged and tracked. Our results suggest that *in vitro* tumoroid models grown on 3D printed dECM can be used as a powerful tool in tissue engineering and disease modelling, to make progress towards understanding cellular behavior and drug responses.

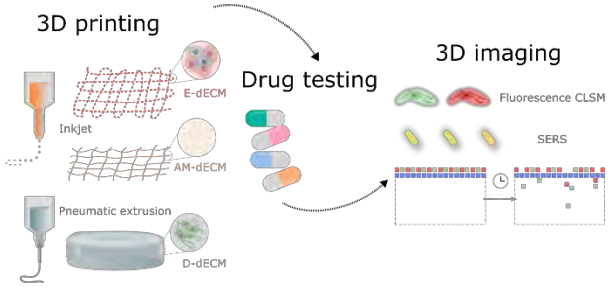

Figure 1. Biofabrication of dECM-based 3D models for drug testing applications.

Keywords: dECM, 3D models, bioprinting, cancer, bioinks.

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Surpassing Substrate–Enzyme Competition by Compartmentalization

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Enzyme compartmentalization is one of the main strategies exploited by nature to create physically separated chemical environments that allow simultaneous enzyme reactions within the cell metabolic networks. However, designing nanostructured architectures that mimic cellular compartments remains a challenge when two competing enzymes must work simultaneously over the same substrate. Herein, we develop a method to fabricate soft hybrids (Capsules) that physically separate two oxidoreductases that compete for NADH with greatly different kinetics. The less competitive enzyme is encapsulated into polymeric capsules capable of recruiting NADH and then assembled on porous agarose microbeads where the most competitive enzyme is immobilized. As a result, this functional hybrid enables the simultaneous action of two competing enzymes in the same reaction media, which would otherwise be impossible in a non-compartmentalized system. We demonstrate that substrate recruitment is a powerful approach to building up enzymatic reaction networks with complex dynamics. Finally, integrating this compartmentalized system into a model cell-free biosynthetic cascade, we transform vinyl acetate into (*S*)-β-hydroxybutyrate with a 2 times higher titer than the non-compartmentalized free system. The proposed strategy can be generalized to produce compartmentalized cell-free biosynthetic pathways and multienzyme cascades where enzyme competition is an issue.

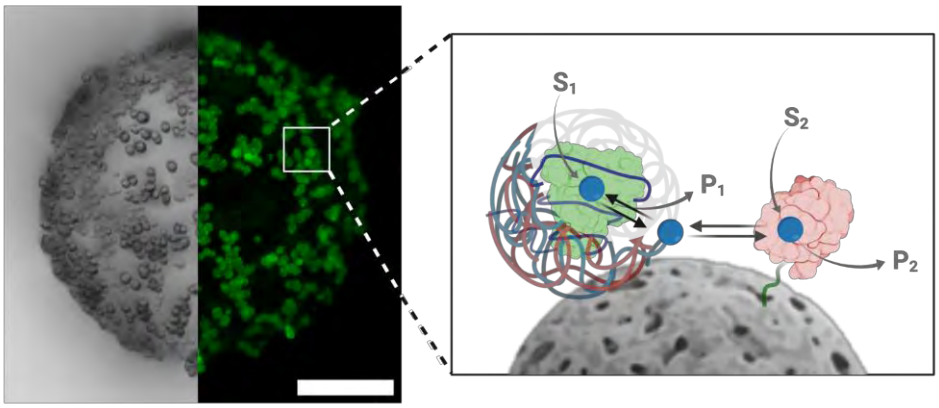

Figure 1. Morphological characterization of the soft enzymatic hibrids immobilized onto agarose microbeads through ESEM and fluorescence confocal z-stack imaging. Schematic representation of two-enzyme system suffering from substrate−enzyme competition

Keywords: Encapsulation, Biocatalysis, Immobilization, Microparticles,

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Scalable enzymatic synthesis of enantiopure β-hydroxy esters and their subsequent polymerization

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INTRODUCTION

Nowadays, polymer science is finding alternatives to produce plastics in a green way, using materials from renewable sources, called bio-based polymers. [1] A polymer that belongs to the group of bio-based polymers is the PHB, polyhydroxybutyrate. PHB is a naturally occurring polyester discovered by Maurice Lemoigne, who isolated and characterized it in 1925. The predominant method to synthesize PHB is by microorganisms under physiological stress as an energy storage system. While this process is interesting on the lab scale, its industrial implementation has not been demonstrated. [3]

In this project, we will synthesize in lab-scale a β-hydroxy ester, which is the repeating unit of the PHB polymer for the subsequent synthesis of the polymer. For the first process, a heterogenous self-sufficient biocatalyst was used, which is able to do an asymmetric reduction from highly abundant β-ketoesters to β-hydroxy esters. [2] In the second step, the β-hydroxy ester will be used for polymer synthesis. For it, the obtained product will be modified by an organic path in order to obtain a 7 member ring. And this molecule will be used to do ring opening polymerization (ROP). In thhis way, we will merge biocatalysis and polymer chemistry to access more sustainable plastics through an enzymatic synthesis of polymer building blocks.

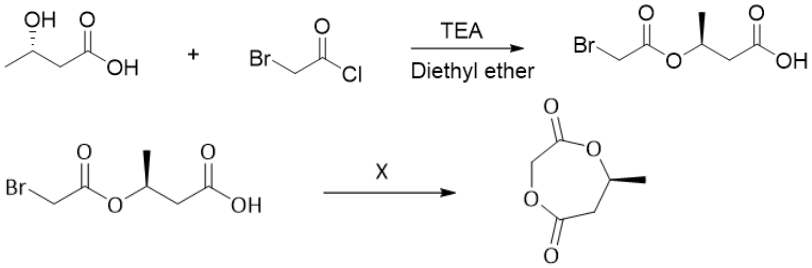

Figure 1. The synthesis path for the 7-member ring

EXPERIMENTAL

For this project, various methods and procedures were used, starting from the basics of organic chemistry, enzyme production and immobilization procedures were used. To characterize the obtained products, various techniques were used, such as microplate spectrophotometer, gas chromatography (GC), nuclear magnetic resonance (NMR), matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), gel permeation chromatography (GPC).

Keywords: biocatalysis, polyester, organic synthesis.

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Determination of glycan profiles on biomarkers by Surface-Enhanced Raman Scattering

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Glycosylation, a fundamental biological process, alters proteins during synthesis in the endoplasmic reticulum and Golgi apparatus. Around 70% of human proteins undergo this modification. Glycosylation's complexity arises from glycan synthesis machinery and its sensitivity to pH, ionic strength, cell interaction or hormones. This sensitivity has diagnostic and monitoring value for diseases like cancer through glycan profiles, distinct in healthy and diseased cells [1].

Traditional methods to profile glycans have limitations, such as antibody dependence, lack of single biomarker glycan information, destructive sample preparation and/or photobleaching using fluorescence probes [2]. To address these, we carry out a sensor combining Quartz-crystal Microbalance with Dissipation (QCM-D) and Surface-Enhanced Raman Spectroscopy (SERS). QCM-D offers real-time, quantification and label-free detection with high sensitivity allowing the use of nucleic acid aptamers as capturing ligands to enhance specificity and avoid cross-reactions [3]. SERS enables molecular detection at low concentrations without photobleaching, aiding multiplexed glycosylation pattern analysis [4].

This integrated setup aims to provide comprehensive information on biomarker concentrations and glycan profiles in clinical samples, aiding early disease detection and monitoring. We work with the aptamer against the Prostate-specific antigen (PSA). PSA is a widely used biomarker to detect and potentially monitor prostate cancer patients. However, PSA tests still lack specificity to distinguish between inflammation events or prostate cancer. Additionally, the determination of cancer-associated glycan modifications is expected to assist in a more precise cancer diagnosis.

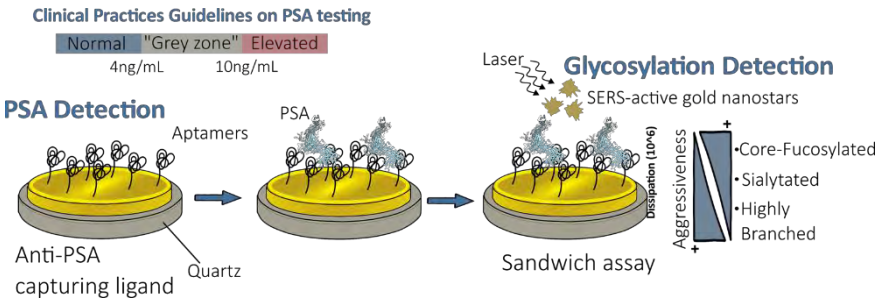

Figure 1. Schematic representation of the diagnostic nanoplatform.

Keywords: Glycosylation, Prostate specific antigen, SERS, QCM-D.

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Chemoenzymatic Oxidation of Diols Catalyzed by Co-Immobilized Flavins and Dehydrogenases

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Enzyme driven oxidations catalyzed by alcohol dehydrogenases rely on the in-situ NAD(P)$^+$ regeneration. A wide variety of chemoenzymatic and fully enzymatic methods have been reported over the last 30 years to integrate cofactor regeneration in biocatalytic oxidations [1]. However, most examples are limited to homogeneous systems where the reuse of both enzymes and chemical catalysts is challenging. In this work, we co-immobilize an alcohol dehydrogenase from *Bacillus stearothermophilus* with a flavin derivative (FMN), which performs as an organocatalyst that oxidizes NADH back to NAD$^+$. This latter oxidized cofactor is sequentially utilized by the alcohol dehydrogenase to oxidize 1,w-diols [2]. Remarkably, the immobilization chemistry of FMN determines its efficiency to oxidize NADH and, unlike in its free state, the immobilized FMN can recycle NAD$^+$ in dark. This is possible because the support where both enzyme and FMN are immobilized also captures NADH, making the electron transfer from the substrates to the cofactors more efficient. This work illustrates how the co-immobilization and confinement of bio and chemical catalysts on solid materials (heterogeneous phase) enable chemoenzymatic cascades that are precluded in solution (homogeneous phase).

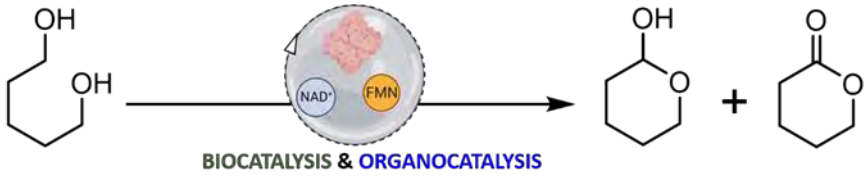

Figure 1. Graphical abstract of the alcohol oxidation reaction.

Keywords: Heterogeneous catalysis, Hybrid biomaterials, Flavins, Dehydrogenases, NADH/NAD$^+$.

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Valorization of Biological Waste from Insect-based Food Industry: Assessment of Chitin and Chitosan Potential

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Edible mealworms can be farmed to produce high-quality nutrients and proteins, useful as ingredients in human and animal foods. During this process biological waste is produced. This work explores the usage of the biological waste as source to produce chitin and chitosan with different potential applications. Different waste fractions were processed, and the feasibility of chitin isolation was assessed. Chitosan was derived, and films were fabricated and tested for intended uses. Data indicate that biopolymers with different properties can be obtained from multiple biological waste fractions. All samples show antibacterial activity, while chitosan films derived from molt show interesting properties for packaging purposes. Films also trigger the expression of anti-inflammatory phenotype markers in macrophage cells, which may be useful for tissue engineering implantation purposes. Altogether, biological waste from insect farming can be used to extract chitin and chitosan with different properties, and therefore, suitable for different applications.

Keywords: *Tenebrio molitor*, chitin, chitosan, films, biopolymer, packaging, biomedicine.

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Combined Surface-Enhancement of Infrared and Raman Signals With Gold Antennas

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Infrared and Raman spectroscopy are powerful biochemical analysis tools as they extract chemical information about samples in a fast and label-free manner. The two techniques are complementary and thus the combination of them yields a more complete picture of the sample. However, both techniques have extremely low sensitivity and thus require enhancement of the signal, which can be performed via technique-specific gold plasmonic antennas.[1] The substrates used for surface enhanced Raman scattering (SERS) have a length scale of tens of nanometers while surface-enhanced infrared absorption (SEIRA) substrates have dimensions of micrometers. The challenge of combining SEIRA and SERS lies in the combination of these length scale elements, while minimizing compromises in the efficiency of both methods.[2]

In this work we present a combined SERS and SEIRA substrate based on infrared antennas produced by electron-beam lithography and metal evaporation, which act as a SEIRA substrate. The key idea behind this approach is to use the IR resonance of a long metal nanorod, while simultaneously enhancing the field enhancement at visible frequencies by the nanospheres that coat the nanorod. As a proof-of-principle demonstration, these antennas were used to detect the cellular metabolite adenine. We functionalize these antennas with SERS resonant nanoparticles which enables the simultaneous detection of adenine with SERS and SEIRA at the same location.

Our results show that SEIRA rods produced with electron-beam lithography functionalized with gold nano-spheres are a suitable substrate for combined surface enhancement of infrared and Raman spectroscopies. Furthermore, our results and simulations indicate that the location of the molecule within the combined sensor and thus the method of assembly are paramount to the efficiency of the sensor.

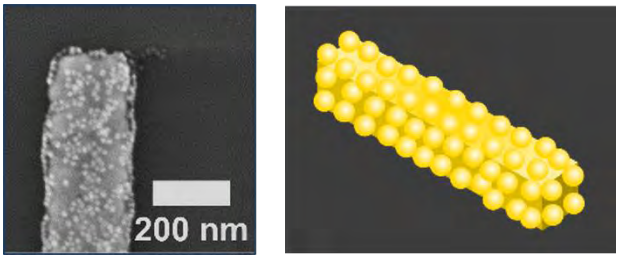

Figure 1. Left: SEM image of a SEIRA antenna functionalized with nanoparticles. Right: schematic image of the sensor concept.

Keywords: Surface enhancement, Infrared, Raman, SERS, sensor

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Tuning the dimensionality of supramolecular materials through the design of peptide-protein co-assemblies.

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Peptide-based supramolecular assemblies have been widely studied for their inherent ability to self- organization, making them useful for applications in nanotechnology.[1] Combining these structures with other molecules opens a new perspective for the design of complex functional materials.[2] Here, we aim to build complex architectures combining a versatile engineered protein, which has potential applications in bioelectronics and nanotechnology, with self-assembled fibers.[3] For this purpose, the protein is modified to incorporate one or two peptides in one or both termini, respectively. This modification will drive the co-assembly with the fibers, resulting in one-dimensional co-assemblies when the protein has one peptide, and an extended network on a second dimension when the protein has two peptides, acting as a fiber cross-linker. The material design was carried out by screening peptide sequences using molecular dynamics (MD) simulations to optimize their co- assembly with the engineered proteins. The resulting architectures were experimentally characterized to prove the change in dimensionality with the number of peptides linked to the protein. The combination of the experimental and computational techniques provided a better understanding of each component in the system, facilitating further rational functional modifications. Therefore, a versatile hybrid material has been created using biocompatible building blocks without introducing any artificial chemical groups or bonds. This material can be customized to suit specific needs in the fields of bioelectronics and biomedicine.

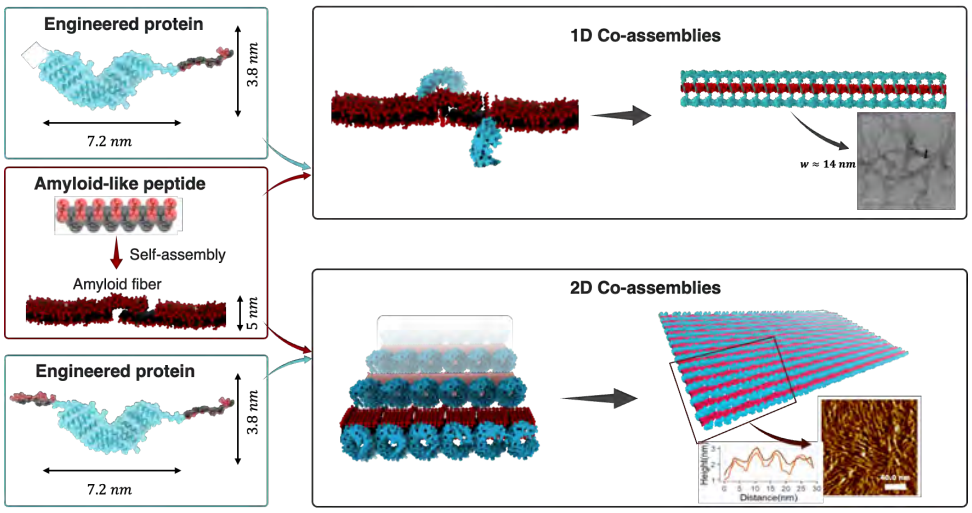

Figure 1. *Rational design of 1-Dimensional (1D) and 2-Dimensional (2D) hybrid materials.* Amyloid fibers co-assemble with modified proteins, with one or two peptides incorporated in one or both termini, resulting in 1D or 2D hybrid materials.

Keywords: Fibers, protein design, self-assembly, molecular dynamics, nanotechnology

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Mechanistic investigation of growth on gold nanorods synthesized with chiral inducers

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** funding from MCIN/AEI/10.13039/501100011033 through grant no. PID2020-117779RB-I0*

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Keywords: chiral, plasmonics, nanorods, circular dichroism

A deep understanding of the underlying growth mechanisms regarding the synthesis of chiral nanomaterials is vital to efficient engineering of viable products. This understanding remains remarkably incomplete, even as the chiroptical signatures yielded from chiral nanomaterials become ever more intense. The optimization of chiral gold nanomaterials in this way holds great potential for the advancement of numerous applications including the fabrication of tunable metamaterials,[1] molecular sensors,[2] and systems for immunotherapy.[3]

Novel chiral molecule LipoCYS is a versatile inducer that can potentially bridge and expand our understanding of a known pair of mechanisms of chiral growth. At relatively low concentrations of LipoCYS, the final chiral morphology descends from the initial symmetry of the seeds[4-6], generating geometrically twisted gold nanorods. At high concentrations of LipoCYS, the formation of NPs with well-defined helical wrinkles, typically obtained through micelle-directed growth[7-8], is observed (see Figure 1). Aided by thorough characterization by electron tomography, LipoCYS was used to investigate the growth of plasmonic chiral nanomaterials and explore the relationship between nanoparticle structure and the corresponding chiroptical signature. Such knowledge can inform the design of chiral inducers and the development of tunable chiral plasmonic nanomaterials.

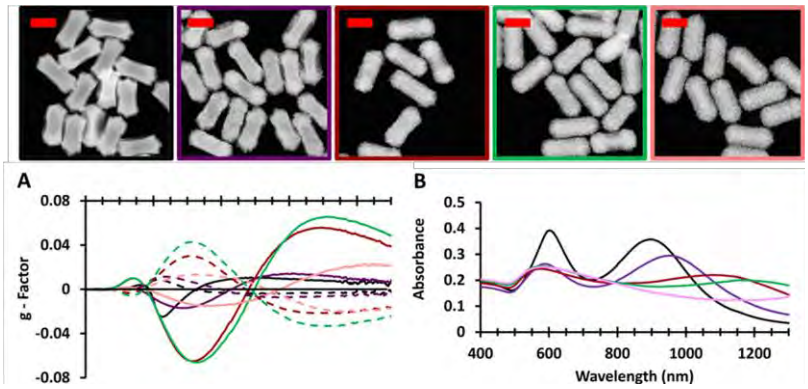

Figure 1. A,B: Circular dichroism (g-factor) spectra (A) and absorbance spectra (B) for chiral AuNR colloids prepared using different [LipoCYS], as labeled. Solid and dashed lines correspond to results from particles synthesized with the (R) and (S) enantiomers of LipoCYS, respectively. Shown above are HAADF-STEM images of chiral particles synthesized with (R)-LipoCYS; scale bars = 100 nm; image outlines are color-coded to match legend of the plots.

Additionally, kinetics experiments were designed to track the chiral growth during the course of reactions prepared with a variety of chiral inducers, to investigate the corresponding mechanisms mentioned above. This time-resolved approach is vital to understanding the relationship between perceived structure and observed chiroptical signature of chiral products.

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Impact of the interlayer distance between graphene and MoS_2 on Raman Enhancement

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Two-dimensional materials and their van der Waals (vdW) heterostructures, particularly graphene and graphene/MoS_2, have attracted intense attention due to their potential application in surface-enhanced Raman spectroscopy (SERS).[1,2] Here, we report how to modulate the SERS response of 2D materials. First, we demonstrate that SERS based on graphene materials is inversely proportional to the functionalization degree. The covalent functionalization interrupts the conjugation of the graphene π-system, inhibiting the charge transfer between graphene and the probe molecule (Rhodamine 6G), thus reducing Raman enhancement. For graphene/MoS_2 vdW heterostructures, the SERS enhancement is dominated by the vdW interaction between graphene and MoS_2. A shorter interlayer distance, with stronger vdW interactions,[3] improves the dipole-dipole interaction and the charge transfer, increasing the Raman enhancement. Moreover, the SERS intensity of graphene/MoS_2 vdW heterostructures varies rapidly when the interlayer distances are less than 0.6 nm, while they vary less at interlayer distances longer than 0.6 nm. This study not only demonstrates the Raman enhancement dependence on the functionalization degree of graphene materials and the interlayer distance in graphene/MoS_2 vdW heterostructures but also opens the door for controlling and predicting SERS intensity based on 2D materials.

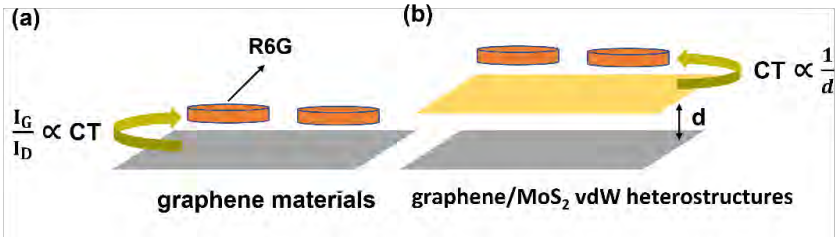

Figure 1. Schematic illustration of the charge transfer process from (a) graphene materials and (b) graphene/MoS_2 to R6G.

Keywords: Two-dimensional materials, Graphene/MoS_2 vdW heterostructures.

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Protein-stabilized nanomaterials as MRI contrast agents: one size does not fit all, but versatility does

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Magnetic Resonance Imaging (MRI) stands as a widely employed diagnostic and imaging method. Nevertheless, it often requires the use of contrast agents to augment the contrast of diseased regions. Yet, the existing contrast agents lack specificity and rely on deviations in anatomy or physiology to create contrast. As a result, there is a pressing demand for the development of more specific and targeted contrast agents.

Protein-stabilized nanomaterials recently emerged as materials of interest in biomedicine due to their biocompatibility, stability, and ease of preparation,[1] presenting advantages over currently available diagnostic and therapeutic agents. Consensus tetratricopeptide repeat (CTPR) proteins are particularly notable for their robustness and mutational permissibility, allowing the introduction of metal coordination sites without substantial structural impact.[2] Moreover, CTPR proteins are modular and can be fused with other targeting or therapeutic peptides or proteins, converting CTPR proteins in a very versatile tool for the development of a contrast agent platform that can be easily and flexibly modified to target and detect a myriad of different diseases.

Herein, we have designed two sets of metal coordinating proteins binding distinct molecular targets, namely Tankyrase and KEAP1. The engineered proteins were used to stabilize iron oxide nanoparticles (IONPs), which demonstrated improved relaxivities. Both TBP (Tankyrase binding peptide) and Nrf2 proteins revealed to bind with high affinity to their target proteins Tankyrase and KEAP1. TBP- and Nrf2-IONPs preserved the binding affinity, indicating that neither the synthesis process nor the presence of the nanomaterials interferes with binding capacity of the protein scaffold.

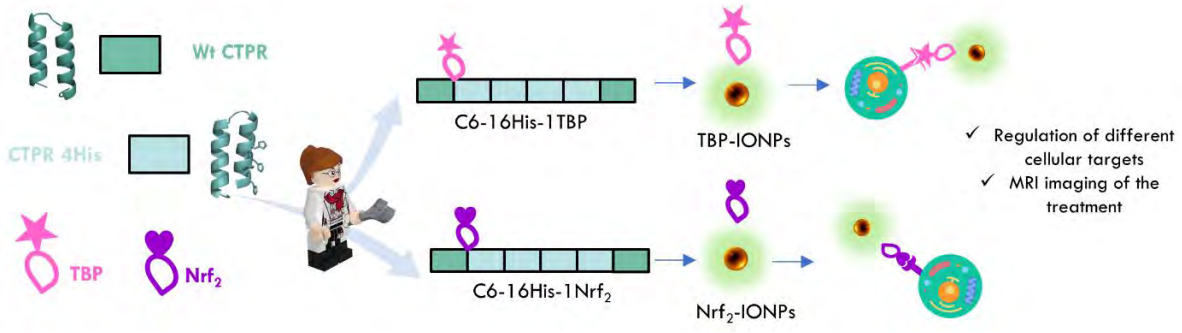

Figure 1. CTPR proteins are used as building blocks to engineer new proteins bearing both metal coordination sites and different binding peptides (TBP and Nrf_2). These proteins allowed the synthesis of Prot-IONPs with different targeting and therapeutic capabilities simultaneously allowing for MRI imaging.

Keywords: CTPR, protein-stabilized nanoparticles, MRI contrast agent, targeted imaging.

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Exploring lung surfactant nanoparticles for pulmonary fibrosis

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In recent years, research has explored the potential of using lung surfactant (LS) as a coating agent of nanotherapeutics and carrier of different drugs for lung diseases, showing promise in enhancing drug distribution, delivery to alveolar cells, and reducing lung inflammation.[1,2] However, pharmaceutical standards and controlled physicochemical properties pose a challenge due to formulation heterogeneity. In response to this challenge, our research proposes the use of hydrodynamic flow focusing (HFF) microfluidic technology, to enable the fine-tuning of nanoparticle size and polydispersity while preserving the chemical composition of LS.[3] Moreover, we demonstrate that this technique favors the encapsulation of the antifibrotic drug Nintedanib and its release in different *in vitro* and *in vivo* models of pulmonary fibrosis (PF). Various fibrotic markers were evaluated to study the therapeutic effects of reducing cell migration, proliferation, and excessive matrix deposition.[4] Additionally, an *in vivo* study using computer tomography (CT) was carried out to non-invasively evaluate fibrosis progression. Preliminary results from the CT combined with histological analysis, indicate the potential of these LS nanoparticles to inhibit or even reverse PF, positioning them as a promising antifibrotic treatment.

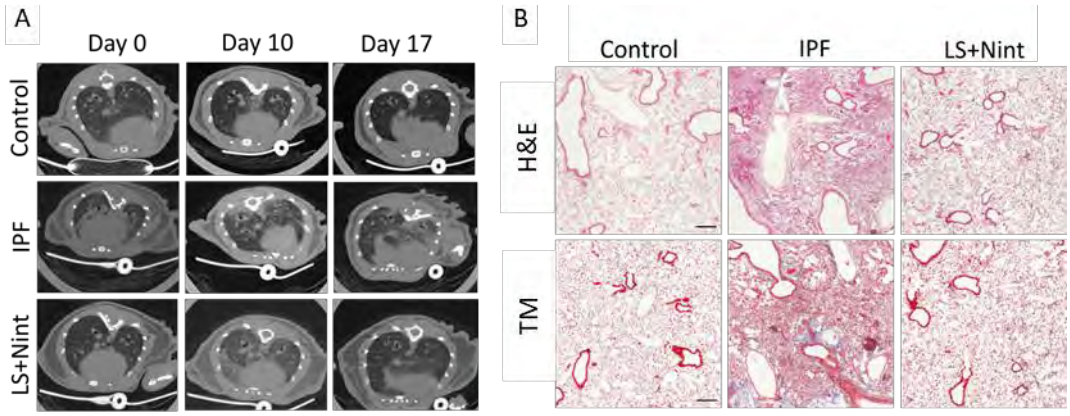

Figure 1. (A) In vivo respiratory-gated CT of lung disease progression and (B) histopathology after the treatment.

Keywords: Lung surfactant, microfluidic synthesis, idiopathic pulmonary fibrosis.

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Evaluating PEG coating of antifibrotic nanoparticles for pulmonary delivery

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Drug encapsulation within nanocarriers and local administration to the lungs have the potential to mitigate the side effects associated with existing treatments for idiopathic pulmonary fibrosis (IPF) as pirfenidone [1]. Pulmonary delivery faces challenges due to the different lung defenses against inhaled pathogens and particles. In this context, the role of polyethylene glycol (PEG) in pulmonary administration is controversial. While PEG is recognized for its antifouling and enhanced mucus penetration, it can also trigger negative immunogenic responses when conjugated to different molecules and nanocarriers [2].

This study aims to investigate the use of lipid nanoparticles with PEG (LP-PEG) or without PEG (LP) to examine nano-bio interactions and determine the most efficient nanocarrier for lung delivery, ultimately improving the therapeutic effect of pirfenidone. The results indicate that PEG enhances mucus penetration but has no significant effect on lung surfactant corona formation or drug release after incubation with native lung surfactant. In vitro experiments demonstrate that PEG reduces cellular uptake however, both liposomes inhibit fibroblast differentiation into myofibroblasts, as well as cell migration and proliferation. Biodistribution experiments in mice reveal that both types of liposomes can reach deep lung regions and remain there for up to 6 days favoring sustained drug delivery. Finally, in a mouse model of bleomycin-induced pulmonary fibrosis, both liposomes attenuate fibrosis progression by reducing collagen deposition and inflammation.

In summary, this study suggests that the inclusion of PEG in inhaled nanomedicines should not be considered a universal requirement, and its incorporation in clinical formulations should be approached with caution due to its potential immunogenicity.

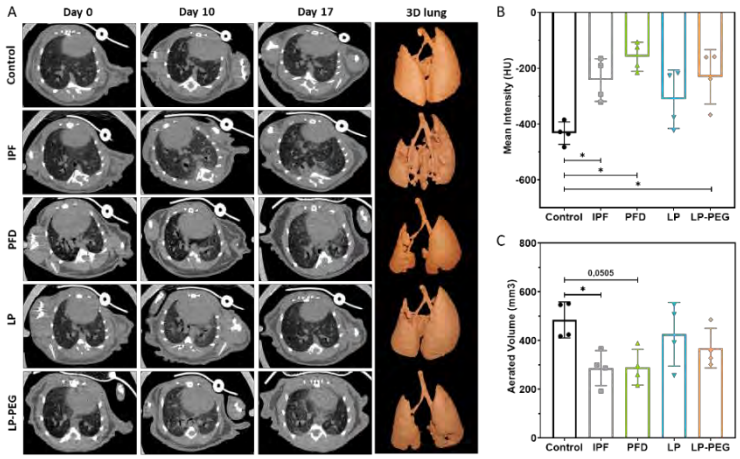

Figure 1. In vivo respiratory-gated CT of IPF progression (A). Quantification of the mean intensity (B) and the aerated volume of the lung (C).

Keywords: PEG, idiophatic pulmonary fibrosis, pirfenidone

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Low dimensional carbon materials as novel multifunctional platforms for biological applications and medical imaging

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Carbon Dots (CDs) are quasi-spherical nanoparticles (<10 nm), made of carbon, nitrogen, and oxygen, owning excellent luminescence properties, water solubility, high biocompatibility, tunable surfaces available for post-synthetic bio-conjugations.[1] Magnetic Resonance Imaging (MRI) is the diagnostic tool of choice for the evaluation of many diseases. Currently, the clinically used contrast agents (CA) are Gadolinium-based complexes that function by catalytically relaxing water protons. On the other hand, they produce side effects due to the gadolinium leaking toxicity. An emerging class of CA are Mn-based compounds as Manganese is less toxic and eco-friendly than gadolinium. Here, we employed fast microwave-assisted hydrothermal reactions to generate luminescent and stable CDs doped with gadolinium (GdCDs)[2] and Manganese (MnCDs) as multimodal probes for MRI. They can retain high relaxivity, show low-toxicity, and good stability in a biological environment.[3] Importantly, they show stable longitudinal relaxivity up to 7 days. Confocal microscopy, ICP-MS analysis and T_1-weighted images on cell pellets confirm cellular permeability of GdCDs, which work as safe and potentially effective CA in *in vivo* studies. Simultaneously, fluorinated MRI agents are another promising challenge in the field of medical imaging. ^{19}F has a high NMR sensitivity and its MRI signal-to-noise ratio (SNR) is about 89% of ^{1}H per nucleus.^{19}F MRI shows lack of background, but generally requires perfluorinated molecules to get a great SNR. Usually, they are not water soluble and are used as nano emulsions. Fluorine doping of CDs is a strategy to get a multimodal probe for MRI.[4] Our goal is to obtain a not toxic, water-soluble fluorinated CA characterized by a significant SNR.

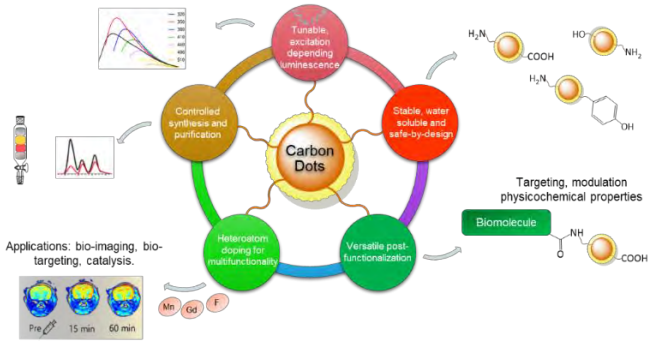

Figure 1. Carbon Dots as multimodal platform with controllable optical and magnetic properties for bio-imaging, bio-sensing, and catalysis.

Keywords: Carbon Dots, MRI, Gadolinium, Fluorine, Manganese

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Synthesis of quantum dots based on novel materials for single photon light-sources

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The environmental monitoring of potentially hazardous substances, such as chemicals or pharmaceuticals, represents a great public interest [1]. Nevertheless, area-wide measurements are a major as the necessary measuring technology to detect such substances in concentrations of ng/l has only been available in laboratories. There is therefore an urgent need for easy-to-use measurement technology to detect potentially hazardous substances in water.

The hypothesis of this work to develop and provide for the first time a non-dispersive technique of a spectrometer as a nano-(opto)electronic system with an electrically pumped single photon source and a highly efficient single photon detector as innovative new systems building blocks with enhanced performance for the measurement of lowest absorptions. The single photon source is based on two nanocomponents, QDs [2,3] as the actual photon source, for which the emission wavelengths are to be extended into the infrared range, and the DNA origami. DNA origami is used for place the single QDs on respective electrode structure, thereby isolating them for the direct formation of single photon sources. Both nanocomponents are combined into the QD-DNA origami hybrid through self-assembly to facilitate integration into a wafer-level QD-LED layer stack. To achieve this, it is necessary to synthesize the QDs with emission wavelengths in visible (Vis) and near infrared (NIR) spectrum region and optimize of QDs-DNA hybrids.

In this work, we synthesized eight types of the non-toxic $AgInS_2$ (AIS) core and AIS/ZnS core/shell QDs using the hot injection synthesis approach [4]. These QDs are characterized by high value of the photoluminescence quantum yield (up to 80%) and luminescence in the Vis spectrum range from 470 to 850 nm, with a NIR maximum at 700 nm. The TEM and DLS analysis showed possibility to change the size of the nanoparticles from 1.5 nm to 35 nm by changing the synthesis conditions. The buildup of the ZnS shell to the core AIS QDs leads to shift of the maximum band position to the shorter wavelength region by passivation of the core defects states. Stabilization of the QDs with glutathione tripeptide leads to appearance of electroluminescence performance. It was also concluded, that the slower injection of the Ag and Na precursors during the synthesis leads to shift of the maximum band position to the red region by 50 nm compare to the typical AIS QDs.

In the next step, we will attach the QDs centrally within the free space of the DNA origami frame, aided by single stranded DNA extruding at specific spots on the DNA origami. By functionalizing QDs with complementary ssDNA strands we can conjugate the QDs to the DNA origami, creating the QD-DNA origami hybrid. This will ensure the least possible influence of the DNA origami on the optical properties of the QDs as well as the charge transport.

Keywords: Quantum dots, DNA, hybrids, spectrophotometer, absorption.

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Self-Assembly of Peptide Hierarchical Helical Arrays with Sequence-Encoded Circularly Polarized Luminescence

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Self-assembled peptide materials with sequence-encoded properties have attracted great interests. Despite their intrinsic chirality, the generation of circularly polarized luminescence (CPL) stronger than typical small organic molecules with tunable wavelength and sequence-encoded properties based on the self-assembly of simple amyloid-like peptides has not yet been reported. Here we report the self-assembly of peptides into hierarchical helical arrays (HHAs) with controlled supramolecular handedness. The HHAs are formed due to the helical orientational ordering of self-assembled peptide nanofibrils driven by the chiral interactions between peptides and incorporated chiral diamines. The HHAs can emit full-color CPL signals after the incorporation of various achiral fluorescent molecules, and the glum value is 40 times higher than that of the CPL signal from the solutions. The sequence of Fmoc-tripeptides plays crucial roles in regulating the helix direction and CPL properties of films. By simply changing the amino acid sequence of the peptides, CPL signals with opposite handedness can be generated within the HHAs. Moreover, molecular dynamics (MD) simulations demonstrate that the peptide HHAs can provide hydrophobic pockets to accommodate the fluorescent molecules with helical arrangement through strong aromatic stacking interactions, which are responsible for the CPL signals. To our best knowledge, this is the first example that the handedness of CPL signal be controlled directly by simply changing the constituent amino acid sequence, rather than chirality of peptide molecules. This work provided a pathway to construct highly ordered chiral materials, which have broad applications in chiroptical field.

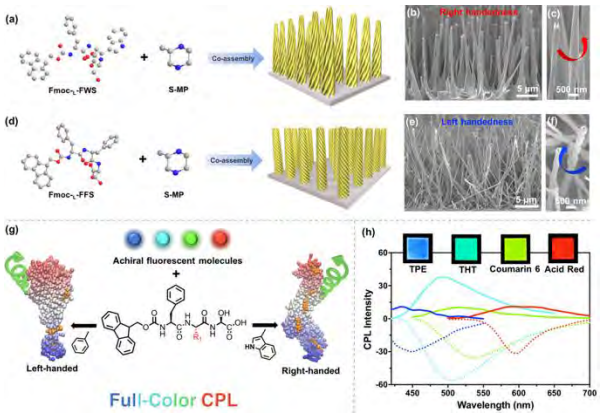

Figure 1 Schematic illustrations of the co-assembly of the (a) Fmoc-L-FWS or (d) Fmoc-L-FFS with (S)-(+)-2-methylpiper- azine (S-MP). SEM images of the HHAs formed by (b-c) Fmoc-L-FWS and (e-f) Fmoc-L-FFS after water vapor annealing at 80 °C for 12 h. (g) Schematic illustrations of full-color CPL based on HHAs. (h) CPL spectra of the Fmoc-tripeptides with different achiral fluorescent molecules.

Keywords: Circularly Polarized Luminescence, Peptide; Chiral Self-Assembly, Hierarchical Helical Arrays, Sequence-Encoded Properties

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Artificial spores as biocatalysts for the upcycling of plastic waste

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With the aim of creating a synergy between whole-cell biocatalysis and enzyme immobilization, artificial spores emerge as enhanced and tuneable biocatalysts. Inspired in nature, we hypothesized that artificial spores could surface as a tool to overcome a wide range of hindered biocatalytic pathways, in addition to grating stability to the systems. In this work, we created artificial spores of Escherichia coli heterologously expressing an alcohol dehydrogenase, coated with ZIF-8[1] and TA-Fe[2] complexes where a co-immobilization of a transaminase and a PETase was performed through different approaches. To validate the formation of the spores, we performed confocal laser scanning microscopy (CLSM) using fluorescence protein labelling (GFP, mCherry) as model proteins to confirm that proteins can be compartmentalized inside and outside of artificial spores. Then, we confirmed that these synthetic spores were metabolically active by a fluoresceine diacetate (FDA) assay by CLSM, in addition to a de-sporulation assay. In this last assay we evaluated the behavior and the ability of each coating to diffuse and perform substrate exchange. Furthermore, to validate the formation of the artificial spores, we performed several characterization techniques such as XPS, ICP-MS, SEM, TEM and AFM, further validating the manufacturing of these spores. Therefore, this study demonstrates the ability to manufacture artificial spores and their viability in biosynthetic purposes.

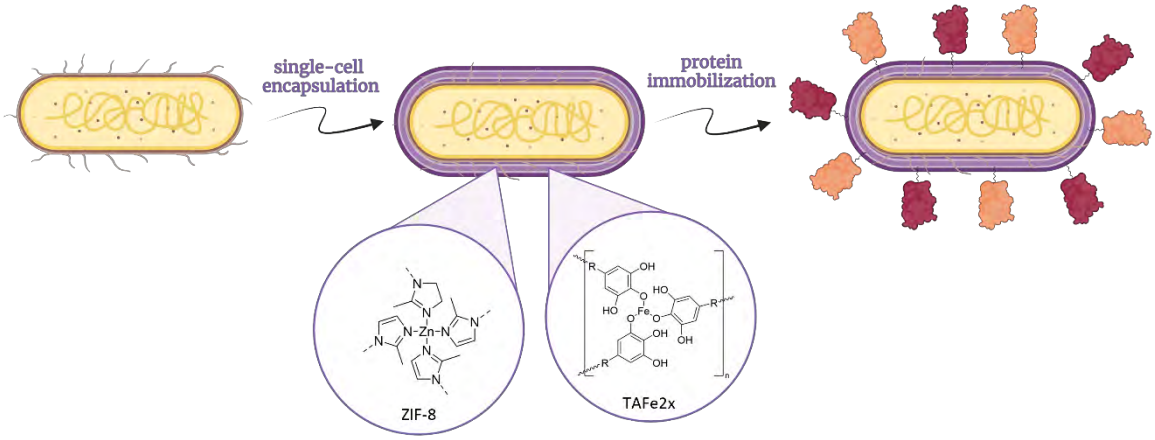

Figure 1. Single-cell encapsulation and protein immobilization scheme.

Keywords: biocatalysis, single-cell, artificial spores, whole-cell biocatalysis, enzyme immobilization.

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Polyallylamine functionalized with dextran for siRNA delivery

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Background. Cationic polyelectrolytes can complex with nucleic acids and deliver them inside cells. Upon endocytosis, polycations with protonable amines induce an osmotic swelling in the acidic environment of the endosomes that facilitates siRNA translocation into the cytoplasm [1]. Polyallylamine (PAH) is an interesting system for intracellular delivery of siRNA. However, positive charges induce toxicological endpoints, limiting the application of complexes of PAH in vivo [2, 3].
Aim. To reduce PAH toxicity, we tested PAH functionalized with dextran. We hypothesize that dextran will shield positive charges, reducing toxicity and/or favoring other endocytic pathways. We have explored the formation of nanoparticles of dextran modified PAH in phosphate buffer and the complexation of the polymers with siRNAs.
Methods. PAH was substituted with different ratios of dextran chain/polymer chain. Dextran functionalized PAH was then complexed with siRNA and characterized via dynamic light scattering and transmission electron microscopy. Gel retardation assay was used to confirm nucleic acids encapsulation. Cytotoxicity was investigated via MTT and XTT.
Results. DLS and TEM proved that less-substituted polymers form nanoparticles in presence of phosphate ions (Figure 1). TEM revealed the different morphology depending on the dextran substitution. Gel retardation assay proved the complexation with siRNA and cytotoxicity studies revealed a lower toxicity of the dextran substituted nanoparticles.
Conclusions. Dextran modified PAH assembles in phosphate buffer and in presence of siRNA. Dextran substitution impacts the dimension and morphology of the nanoparticles. The presence of dextran reduces cytotoxicity.

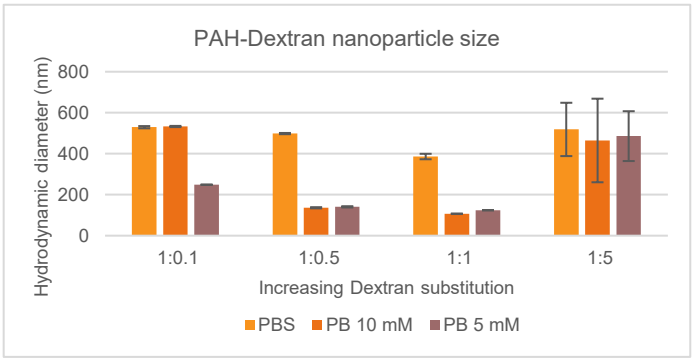

Figure 1. Dimension analysis of dextran substituted PAH nanoparticles via dynamic light scattering.

Keywords: Nanoparticles, polyallylamine, dextran, siRNA, delivery.

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High-throughput immobilization screening for assembling cell-free immobilized enzymatic cascade

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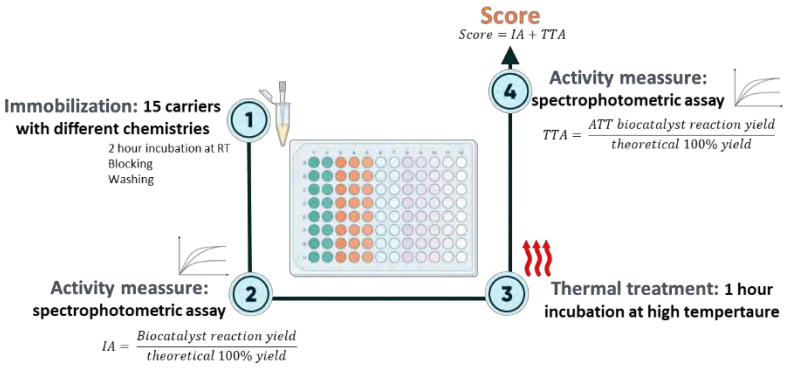
Immobilization is the confinement of an enzyme in space to facilitate its reuse and recovery.[1] Enzymes are composed of different sequences of amino acids; hence, its surface possesses different chemical properties. For this reason, there is no universal protocol for enzyme immobilization. To find the best choice for each enzyme, different immobilization chemistries should be tested. Therefore, the establishment of a protocol that can rapidly test which reactive groups are suitable to obtain an active and stable heterogeneous biocatalyst is of great interest. In the present work we describe a protocol in which 15 carriers with 7 different functional groups can be tested in a 96 well-plate. The best carrier is selected by the highest score, which is calculated considering the initial recovered activity and the activity after the thermal treatment. A screening to immobilize a thiolase (THL), a thioesterase (TES), an alcohol dehydrogenase (ADH) and an aldehyde dehydrogenase (AlDH) was performed. THL was successfully immobilized on a carrier functionalized with aldehyde (GA) groups, TES was on a carrier with a trifunctional carrier with epoxy groups (AgCoBDGE), ADH and AlDH were on a carrier with glyoxyl groups (Gx). These immobilization protocols are being scaled up to prove the efficiency of the screening. The final step will be to immobilize each enzyme in the best carrier and assemble an enzymatic cascade to produce 3-hydroxybutyric acid from vinyl esters as it has been previously performed by soluble enzymes.[2]

Figure 1. Workflow of the plate immobilization screening



Keywords: High-throughput immobilization, immobilization chemistries, enzymatic activity, enzyme stabilization.

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Rational design of all-protein-based conductive system

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New era of wearable devices will be characterized by an increasing presence of components of origin or inspired by biological systems. The possibility of using self-assembling protein-based systems in communication with inorganic nanostructured supports, respectively produced according to bottom-up and top-down techniques, opens the way to new technologies applicable to different research fields such as healthcare, energy, electronics etc. One of the main challenges of the design of new devices is the development of the immobilization technique that also allows the study of the conduction system formed by the protein matrix and the inorganic support. With a rational design approach, the development of a all-protein-based conductive system and its study, on several levels of protein organization, from single protein until thin film structures, may guarantee the proper function of the conductive system.

For this purpose, CTPRs[1] will be used as starting point for their good level of structural stability and inherent biocompatibility, commonly absent when organic or inorganic materials are used. Moreover, the ability to control the aminoacidic sequence provides high variability for the same system in terms of chemical and physical properties[2].

In this PhD project, CTPRs properties, especially the high variability, will be used, by rational design approach, to develop conductive all-protein-based devices controlling every single mechanical, physical and chemical aspect at every level of organization, from single protein to the whole material, by means of modifying only the protein sequence.

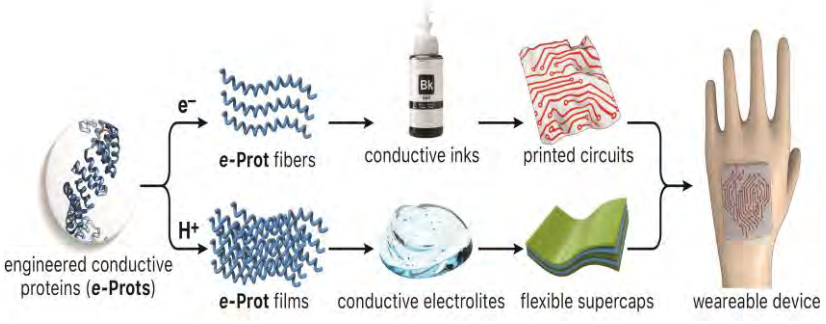

Figure 1. Roadmap of the e-Prot project.

Keywords: protein-based materials, CTPRs, protein conductivity, wearable materials, rational design.

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Synthesis of smooth sub-micron gold nanoparticles

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Despite the significant advances in the colloidal synthesis of metal nanoparticles, the preparation of smooth and monodisperse spherical particles in the intermediate size range (100 – 500 nm) is still challenging. Some applications require stringent conditions regarding monodispersity and smoothness, for example when the particles are to be used as building blocks in self-assembled systems. Existing protocols for the preparation of monocrystalline gold spherical particles are usually limited to sizes below 100 nm, when highly spherical (smooth) and monodisperse particles are required. Therefore, the optimization of such synthesis methods is needed to produce larger smooth and monodisperse particles. We have explored the use of a method for stepwise growth and subsequent etching of the particles, which can lead to sizes up to 200 nm with a good dispersity but can likely be expanded beyond this limit. Firstly, particles of 80 nm are synthesized using a one-shot injection of gold salt in the presence of 10 nm seeds. Then, the particles are further grown using a syringe pump and a shorter etching step. The figure of merit of this method is the low size dispersity and good sphericity of the produced particles up to 180 nm. Moreover, this method has a relatively low reaction time despite the use of a syringe pump, only a total of 1h30 is needed to produce the final 180 nm particles. Preliminary results showed that 160 nm particles can be further grown to produce 210 nm particles. However, the production of these objects requires adjustments to reach high quality.

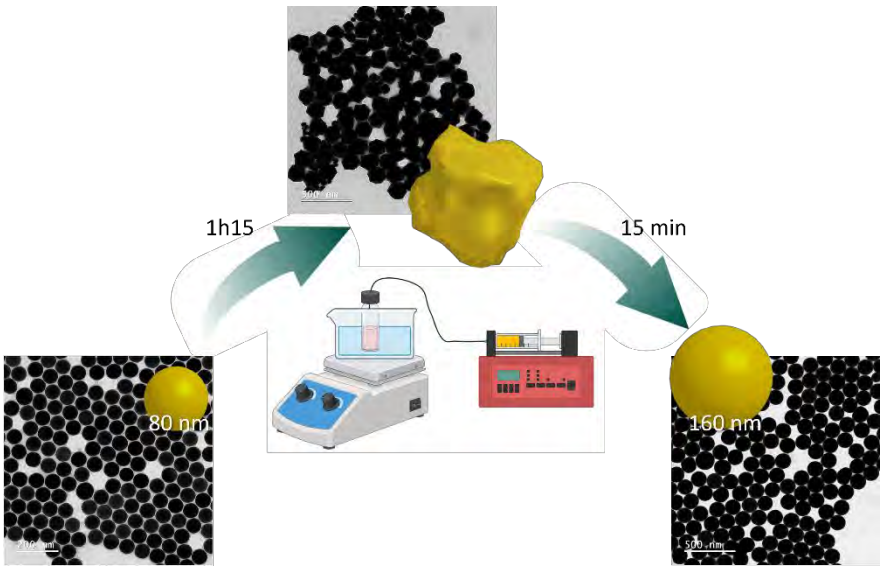

Figure 1. Graphical abstract representing the setup used for the preparation of 160 nm particles from 80 nm.

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Engineering of new enzymes for the development of biocatalytic systems and nanosensors

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Protein engineering constitutes a pivotal field situated at the confluence of biology and chemistry, enabling the deliberate modification and conceptualization of proteins with the aim of attaining particular functionalities. Biocatalysis and nanotechnology stand out as two captivating domains profoundly influenced by the strides made in protein engineering [1][2]. In prior research, an artificial variant of the Consensus Tetratricopeptide Repetition protein (CTPR) was engineered to catalyze a Huisgen (3+2) cycloaddition, resulting in the formation of several diastereoisomers of an unnatural nitroproline ester. This diastereoselectivity was contingent upon the protein's flexibility and the arrangement of a catalytic dyad consisting of lysine and glutamate [3]. The primary objective is to develop nanozymes that integrate coordinated metal complexes, which will serve as Lewis's acids, in place of a functional group within the catalytic dyad. Through computational simulation, the catalytically active amino acid will be replaced with a metallic complex, thereby enabling the establishment of a coordination sphere. The anticipated result is the creation of modified nanozymes proficient in selectively catalyzing reactions towards particular diastereoisomers. The second objective centers on protein engineering and the pivotal use of artificial topoisomerases to drive aptamer-based nanosensors, particularly those utilizing supercoiled DNA. Synthesis of dsDNA will stem from the coding sequence of a defined aptamer inserted in a previously described plasmid, coupled with the production of relevant artificial topoisomerases. Variant selection will be based on expression levels, stability, and specific affinity to their target, critical in obtaining optimal DNA nanosensors.

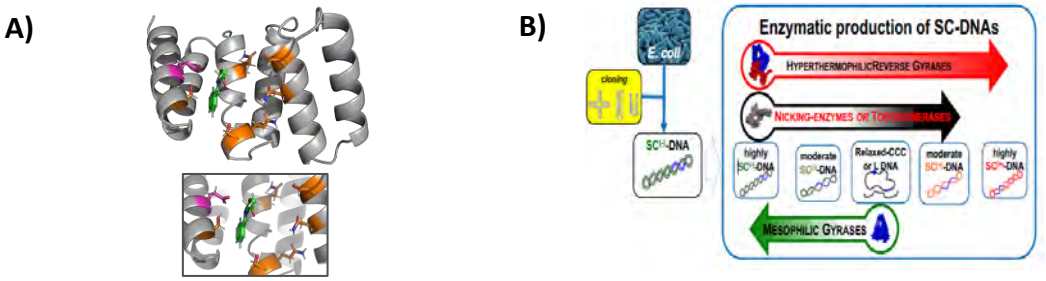

Figure 1. A) Nanozyme biocatalysis. Computational approach to rationally modify the CTPR nanozyme sequence to acquire a specific diastereoselectivity and efficiency of the catalyzed Huisgen (3+2) cycloaddition**. B) DNA-nanosensors.** Possible topology modifications of the nanosensors through topoisomerase activity to monitor conformational transitions of supercoiled (+), (-) and relaxed DNA and their affinity to their target.

Keywords: Biocatalysis, diastereoselectivity, nanozymes, nanosensor, topoisomerase, aptamer

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N-Glycomimetics and exosome glycoengineering targeting immune system lectins as potential cancer therapies

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Exosomes are 40-160nm sized vesicles secreted by most types of cells as vehicles for intercellular trafficking of cellular components including miRNA, mRNA, proteins, lipids, metabolites etc. Their ability to connect distant tissues has stimulated a broad interest in exosomes as a natural non-immunogenic drug and gene delivery vehicles.[1] Considering the importance of glycans on exosome targeting capability,[2] we aim to produce exosomes, as a potential tool for drug delivery in cancer therapy using glycoengineering. The idea is to produce mesenchymal stem cell derived exosomes with covalently attached sialyl LewisX tetrasaccharide, a molecule with high affinity to selectin, a human lectin overexpressed in endothelia of inflamed tissues including tumors.[3] To start, we decided to synthesize sialyl LewisX with anomeric PEG linker (sialylLewisX-PEG-NH_2) via a combination of chemical and chemio-enzymatic reactions from lactosamine. For the chemo-enzymatic part, both a α2−3-sialyltransferase and a α1,3 fucosyltransferase were expressed and purified. After each purification, a protein electrophoresis, and an activity assay by UPLC were performed. Once sialylLewisX-PEG-NH_2 is ready, this compound will be activated as NHS or tetrafluorophenyl activated carbamates for targeting readily accessible lysine residues of exosome surface proteins. We will evaluate the effects of reaction time, buffer pH and reagent excess on the degree of surface functionalization with the help of mainly anti-sialyl Lewis X antibodies, MALDI-TOF MS and Z-potential. Moreover, we will employ lectin-array technology for analyzing surface glycosylation before and after every glycoengineering approach.

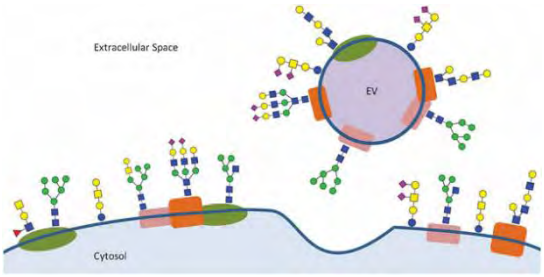

Figure 1. Exosome (EV) and its glycan surface attaching to recipient cell. Extracted from reference [2].

Keywords: cancer, exosomes, glycotechnology, sialyl LewisX, lectin

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SERS sensing in 3D cell cultures

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Drug and therapy discovery against cancer is limited by the default of cellular models able to mimic tumoral growth and tumoral interactions with the environment.[1] Conventional 2D cellular models are suboptimal and, therefore, more complex 3D in vitro models are required to have closer conditions to those inside living beings.[2] To study cellular behaviour, sensitive and non-invasive techniques are needed such as Surface-Enhanced Raman Spectroscopy (SERS) which allows to detect low concentrated metabolites located on or next to metallic nanostructures.[3] Therefore, incorporating these nanostructures into 3D cellular models would allow for in situ detection of metabolites and biomarkers secreted by tumoral cells. Herein, plasmonic nanoparticles are combined with different hydrogels (methacrylated gelatin, methacrylated alginate, methylcellulose, etc.) to obtain hybrid bioinks that could be used for printing sensing materials within a 3D cell model. The main goal of this work is to use the hybrid bioinks to print the sensor inside a decellularized extracellular matrix (dECM) bath containing different cancer cell types to support tumoral cell culture and monitor cell behavior over time (*figure 1*). Preliminary results show that the sensor can be printed inside a dECM matrix able to support and retain the desired cylindrical shape of a tube of the plasmonic hydrogel. Also, the sensor can be printed inside a 3D tumoral model keeping the integrity of the system and allowing cell growth and spreading over time. [4] The sensor allows the SERS detection of different metabolites (4-mercaptobenzoic acid, methylthioadenosine, hypoxanthine, doxorubicin and 6-thioaguanine) inside the dECM matrix. Further characterization will involve in situ SERS sensing of cell-secreted metabolites related to specific biological processes such as tumor progression.

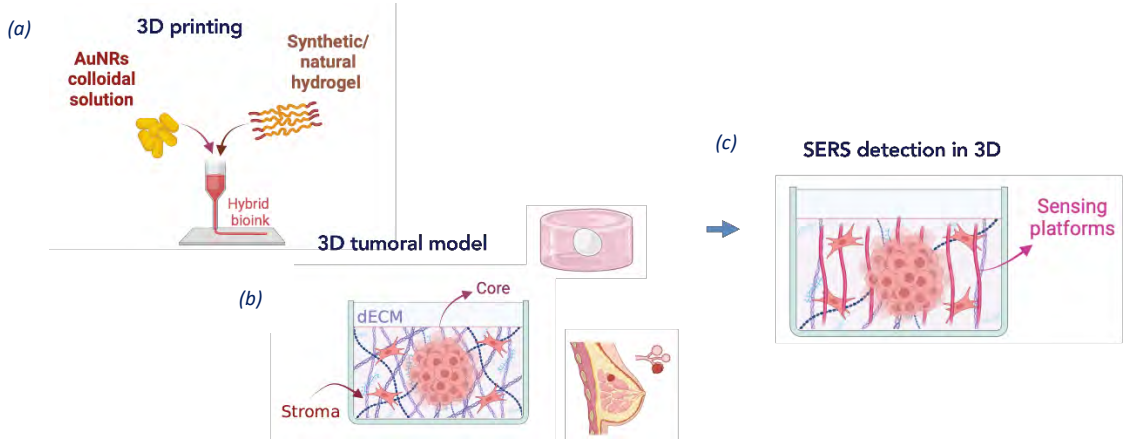

Figure 1 (a) Bioink fabrication; (b) 3D tumoral model; (c) Plasmonic grid for SERS monitoring inside the 3D tumoral model.

Keywords: 3D cellular models, Surface-enhanced Raman Spectroscopy, 3D printing, plasmonic, hydrogels, bioinks.

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Hemoglobin based Oxygen Nanocarriers: An exogenous supply of O_2 for applications in photodynamic therapy

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Photodynamic therapy (PDT), a minimal invasive technique for cancer treatment, is based on treating tumors by generation of reactive oxygen species (ROS) by the activation of O_2 through light pulses[1]. This energy is captured and directed to oxygen by photosensitiser molecules (PS)[2].

One of the main drawbacks of photodynamic therapy in cancer treatment is the limited O_2 content in tumour tissue, which limits the ROS production. The use of hemoglobin (Hb), the natural O_2 carrier in blood stream, could be an interesting approach for oxygen supply to the tumor during PDT[3].

Hemoglobin based Oxygen Nanocarriers (HOBC) were prepared by entrapping hemoglobin in polymeric or protein nanoparticles. It could provide a means to transport oxygen to tumor tissue. Desirable size for tumor penetration and non-immunogenic effect could be below of 500 nm.

Our research is focused on the synthesis of small-size HOBCs that could be applied for tumor targeting. Two different approaches were tested for protein NPs fabrication. **Co-precipitation** with carbonate templates and **polymer complexation**.

Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), UV-Visible Spectra, Circular Dichroism (CD) were applied for an in-deep characterization of physico-chemical properties of our systems.

Particles obtained by co-precipitation method had a size around 300-350 nm, while particles synthetized by polymer complexation get size around 30-50 nm.

In conclusion, the nanosystems designed could be the basis, for the implementation of PDT by exogenous oxygen supply.

Keywords: Hemoglobin, nanoparticles, co-precipitation, complexation, polymer, oxygen

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3D printed & microfluidic models of breast cancer lung metastasis

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Breast cancer (BC) remains a leading cause of cancer related mortality in women world-wide. Lungs represent the second most common site of BC metastasis and onset can highly vary across patients, with some women experiencing metastatic outgrowth up to decades after initial diagnosis (1-3). Emerging evidence indicates that the extracellular matrix (ECM) plays a pivotal role in guiding tumor evolution via a plethora of bioactive molecules and mechanical stimuli (4-5). In this context it remains to be determined how lung ECM stiffness affects metastatic outgrowth of dormant BC cells. Here we synthesize tunable hydrogels for 3D bio-printing as well as microfluidic platforms to mimic the metastatic lung niche. Upon synthesis, norbornene-modified alginate (N-Alginate) and methacrylated gelatin (GelMA) were mechanically characterized by rheology and tuned in the range of the stiffness of healthy lung tissue (0.8-2 kPa) via visible light (405 nm) induced crosslinking. Initially we developed endothelial tubules within these microfluidic platforms to provide physiologically relevant perfusion of nutrients, waste and drugs. Within microfluidic chips, endothelial cells display barrier formation on N-alginate and maintain high viability on GelMA throughout several days of dynamic culture. Moreover, via 3D printing, perfusable channels could be fabricated within GelMA bulk hydrogels using gelatin as a sacrificial ink. Preliminary data show that endothelial cells are retained within the channel and display high viability upon sacrificial ink removal. Together, these approaches provide promising techniques to generate vascularized 3D printed and microfluidic platforms, which can be tuned to match niche characteristics and study metastatic progression.

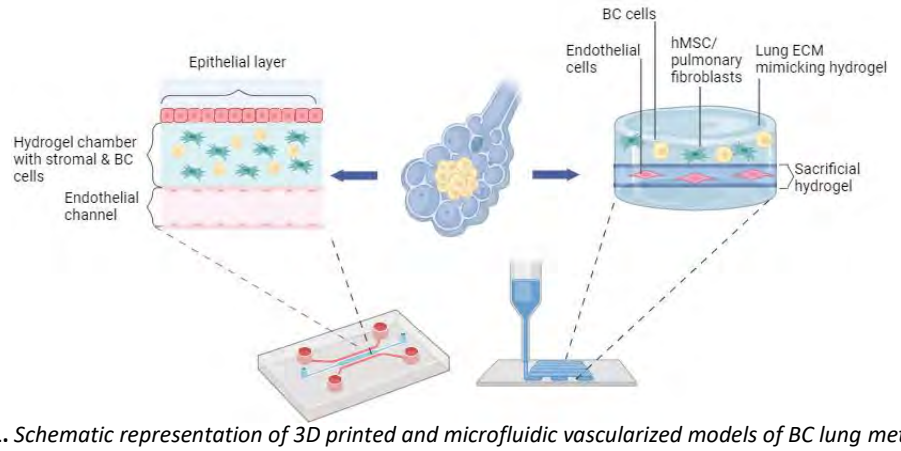

Figure 1. *Schematic representation of 3D printed and microfluidic vascularized models of BC lung metastasis.*

Keywords: breast cancer, lung metastasis, extracellular matrix, 3D bioprinting, microfluidic

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Development of RNA-editing tools through engineering of biomolecule-nanomaterial hybrids for therapeutic approaches

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Transcriptome editing has opened an array of possibilities for new therapeutic strategies, such as the modulation of mRNA splicing using antisense oligonucleotides (ASOs), a therapy already approved for spinal muscular atrophy (SMA)[1]. However, the use of ASOs present some disadvantages, and is important to explore alternative strategies, such as CRISPR-Cas13 and engineered RNA-binding repeat proteins.

The CRISPR-Cas13 system offers the ability to precisely target RNA without sequence constraints. A catalytically inactive Cas13 (dCas13) nuclease forms a ribonucleoprotein complex with a crRNA to bind a specific sequence in a target RNA and modulate its splicing[2]. Protein stabilized gold nanoclusters (AuNCs) are of great interest in biomedicine. They present distinct advantages, including enhanced protein stability, good biocompatibility, fluorescence, and intracellular tracking[3]. In this project, we fuse dCas13 with a consensus tetratricopeptide (CTPR) protein, engineered to coordinate metals, in order to stabilize AuNCs and obtain a protein-nanomaterial hybrid able to modulate splicing.

Furthermore, we investigate RNA-binding repeat proteins, such as the pentatricopeptide (PPR) and the Pumilio and FBF (PUF) families. These proteins can selectively bind specific RNA sequences based on an amino acid code, in with each repeat recognizing a single base[4,5]. To obtain stable scaffolds for RNA editing, we use a consensus PPR sequence[5], and design a *de novo* consensus PUF sequence. We designed both proteins to recognize a specific sequence in *SMN2* mRNA as a therapeutic strategy to correct splicing in SMA.

These engineered proteins efficiently modulate splicing and are promising tool for transcriptome editing in other diseases.

Keywords: splicing, RNA, CRISPR-Cas13, PPR, PUF.

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CNT-based scaffolds as a regenerative approach in animal models of spinal cord injury

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Spinal cord injury (SCI) leads to the loss-of-function of neural tissue, as there is currently no treatment that allows full recovery. 3D scaffolds mimic tridimensional tissue structure, while carbon nanotubes (CNTs) have emerged as promising conductive materials to interface with electrically active tissues due to their unique electric and mechanical properties. We aim to use CNT containing hydrogels as therapeutic implants in the SCI lesion site to re-establish the neuronal connections and regain the functionality of the spinal cord. Firstly, *in vitro* assays will be performed, culturing cells inside CNT-doped polymer 3D scaffolds. Cell viability will be assessed by LDH assays. Cell morphology, attachment, differentiation, and maturation will be studied by immunohistochemistry, together with calcium imaging to measure neurotransmitter release. Other cellular features will be assessed by SEM and LSCM microscopy. The scaffolds showing best results will be then used in the following *in vivo* implantation experiments. Three experimental groups will be formed: control animals, SCI rats with CNT-free hydrogels and SCI rats with CNT-containing hydrogels. SCI animal models will be generated by performing a clean cut of the left hemicord at the T8 vertebra level. The restoration of the locomotor function will be evaluated by behavioural tests (BBB and Horizontal Ladder tests), while the anatomical structure of the spinal cord will be analyzed by MRI. After sacrifice, regeneration at the lesion site will be evaluated by immunohistochemistry techniques. This work will evaluate spinal cord recovery for both untreated control animals and those implanted with our CNT scaffolds.

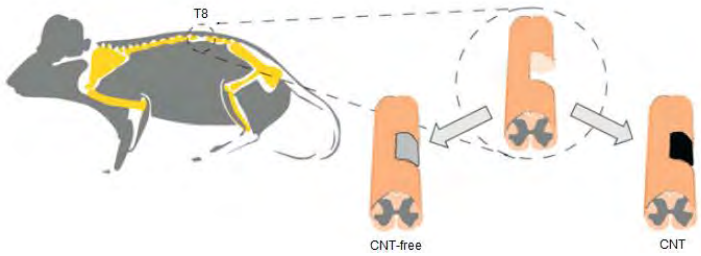

Figure 1. Schematic representation of the *in vivo* experimental design.

Keywords: Carbon Nanotubes, Spinal Cord Injury, Scaffolds, *In vivo*, Neuroregeneration

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Polyallylamine-plasmid complex: A physico chemical study

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Plant genetic engineering for phytoremediation approaches is still poorly explored due to difficulties in genetic manipulation of non-model-plant species, often used for this approach. A promising strategy for enhancing phytoremediation is to generate plants with enlarged root system and an increased absorption capacity, obtained by modifying genetic traits that regulate root development.

In plant genetic engineering, nanomaterials have been used as vehicles for the delivery of plasmid DNA and short-interfering RNA (siRNA) to plants through the infiltration of leaves. However, so far there are no works reported about the use of polymeric nanoparticles for generating non-food crop with enhanced abilities for phytoremediation purposes.

Recently, in animal cell studies, modified polyallylamine hydrochloride (PAH) polymeric nanoparticles have found applications as vectors of genetic material. These cationic polymers interact electrostatically with negatively charged nucleic acids[1,2,3]. Polyallylamine phosphate (PAN) polymeric nanoparticles have been used successfully as vectors for siRNA. These complexes enter the cells by endocytosis and are directed to lysosomes where the acid pH induces the dissociation of the complexes, leading to the release of siRNAs in the cytoplasm[4].

In this work we have studied the complexation of nucleic acids for plant genetic with PAH molecules with different modifications: oleic acid and dextran, aiming at having a more physical insight on the properties of the polyplexes formed. We have studied the process of complex formation and characterized the polyplex nanoparticles by a combination of techniques: Fluorescence Correlation Spectroscopy (FCS), Dynamic Light Scattering (DLS), Small Angle X-ray Scattering (SAXS) and Transmission Electron Microscopy (TEM).

Keywords: Plant genetic engineering, polymeric nanoparticles, polyallylamine hydrochloride, dextran.

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Evaluation of Iododiflunisal dose-response on amyloid-β plaques and neuroinflammation in the 5xFAD Alzheimer's disease mouse model by PET imaging.

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β (Aβ) aggregates. Transthyretin (TTR), a tetrameric Aβ-binding protein, has been shown to reduce Aβ toxicity. Small molecules that stabilize TTR's tetrameric form, such as iododiflunisal (IDIF), can enhance its ability to interact with Aβ and may have potential as disease-modifying drugs in AD[1].

Fifty 2 months-old female mice were divided into 5 groups and orally treated with different doses of IDIF: 0, 10, 30, 100 and 300 mg/kg/day. Treatment efficacy was assessed by longitudinal changes in the deposition of Aβ at ages=2 and 8 months determined using PET-[^{18}F]florbetaben. Further, neuroinflammation was assessed with the TSPO PET radiotracer [^{18}F]DPA-714 at 8 months of age.

Plasma concentration of IDIF was determined monthly by UPLC-MS/MS analysis, and it shows a good correlation with the administered dose. However, Standard Uptake Values relative to the cerebellum (SUVr) of [^{18}F]florbetaben in cortex and hippocampus increased from age=2 to 8 months, irrespective of the treatment received. The uptake of [^{18}F]DPA-714 at 8 months of age showed significantly lower levels of neuroinflammation in hippocampus, cortex and thalamus in the IDIF-30 group compared to IDIF-100.

These findings suggest that IDIF is ineffective at reducing Aβ deposition in the 5xFAD AD model after 6 months of treatment. However, previous results in a less aggressive model have shown that IDIF can delay the appearance of Aβ plaques[2]. Future studies should evaluate its ability to delay the appearance of Aβ plaques in the early stages of treatment.

Keywords: Alzheimer´s disease, amyloid-β, Positron Emission Tomography imaging, iododiflunisal.

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Magnetic resonance imaging study of myelin in aging and neurodegeneration

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Myelin, a fundamental component of the central nervous system (CNS), plays a crucial role in neuronal function and cognitive processes [1]. With aging and neurodegenerative conditions, such as multiple sclerosis and Alzheimer's disease, myelin undergoes degeneration, leading to detrimental consequences [2]. This project aims to investigate the relationship between myelin, brain connectivity, and cognitive function in animal models of both healthy aging and pathological neurodegeneration. Additionally, it explores the potential therapeutic effects of Clemastine in promoting myelin recovery.

To achieve these objectives, a multi-faceted methodology is employed. Resting-state functional magnetic resonance imaging (rs-fMRI) techniques are developed in mice, allowing for the examination of brain connectivity in various life stages. This is coupled with behavioral tests to assess cognitive functions throughout youth, maturity, and old age in healthy animals. Furthermore, magnetic resonance imaging techniques are utilized to evaluate myelin levels in the brain, validated against histological techniques, thus providing a comprehensive understanding of myelin content in different study groups. The study also investigates changes in myelin levels and brain connectivity in animal models subjected to demyelination and remyelination (cuprizone intoxication animal model), as well as the impact of Clemastine as a potential therapeutic intervention.

The project's findings hold promise for a more profound understanding of the role of myelin in aging and neurodegeneration, with implications for the development of effective therapies. By investigating the relationship between myelin, brain connectivity, and cognitive function, this study offers a comprehensive approach to addressing the complexities of neurodegenerative conditions and promoting healthier brain aging.

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Fluorescent carbon nanomaterials with innovative and multifunctional features for biomedical diagnostic and imaging applications

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Carbon dots (CDs) are nano-sized carbon materials characterized by high water-solubility, high biocompatibility, excitation-dependent photoluminescence behaviour, and high resistance to photobleaching[1]. Besides their synthesis, the application of new purification criteria is useful to achieve more reliable CDs, free from the interference of artifacts. A smart selection of the precursors for CDs synthesis allows to tune their physicochemical properties. Accordingly, there is a growing interest in the synthesis of metal doped CDs to endow the final nanoparticles with peculiar characteristics. To that aim, this project is focused on the one pot synthesis, purification and characterization of Gd(III) doped Carbon dots (GdCDs) as simultaneous positive contrast agents in Magnetic Resonance Imaging (MRI) and cellular staining in Fluorescent Imaging (FI) applications. Recently, GdCDs with high solubility, biocompatibility, and relaxivity properties have been produced to enhance the characteristics of Gd(III) as a contrast agent[2]. In this work, GdCDs has been synthetized from Gd-DTPA complex and β-Alanine via microwave assisted synthesis and purified via filtration, dialysis, and ion exchange chromatography (Figure 1). Using an already formed contrast agent in the synthesis, we aimed at achieving higher stability of Gd(III)-based nanoparticles with respect to previously published works. Moreover, our goal was to obtain a fluorescent system with higher cell penetration and biocompatibility with respect to the lone contrast agent. The so-synthetized and purified GdCDs presented good homogeneity (3.5 - 7.5 nm), high Gd content (22% w/w), suitable longitudinal relaxivity (r_1), and emissive properties, resulting as suitable candidates for combined MRI and FI applications. The *in vitro* and *in vivo* experiments are currently ongoing.

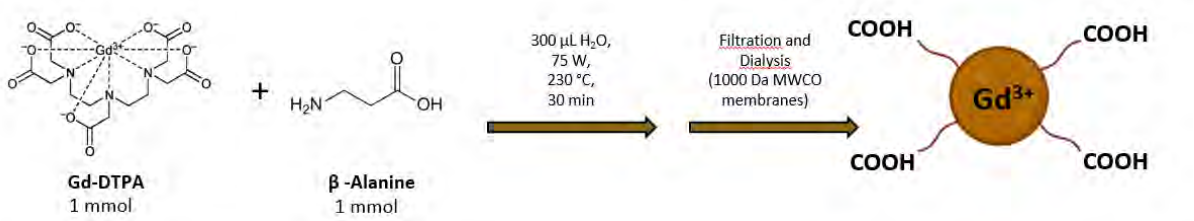

Figure 1. Synthesis of Gadolinium-doped Carbon Dots (GdCDs) with a microwave-assisted method.

Keywords: Carbon Dots, Gadolinium Contrast Agents, Magnetic Resonance Imaging, Nanomedicine, Bioimaging.

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Novel synthesis of Fluorescent Iron-Doped Carbon Nanodots: new insights on medical imaging.

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Magnetic Resonance Imaging (MRI) is one of the most important non-invasive imaging modalities in clinical diagnostics and research. Nowadays, most of the contrast agents used clinically are Gg-based but, lately, they have been associated with organ damage and accumulation in tissues due to Gd leaking. Fe-based contrast agents are emerging as an alternative to Gd-based ones, since they present less associated toxicity and good relaxivity properties. Carbon Nanodots (CNDs) are very appealing materials for biomedical applications, since they show low cytotoxicity, high water solubility, tunable fluorescence emission and abundant functional groups on the surface. Thus, doping CNDs with Fe allows their use as highly biocompatible contrast agents for MRI. Accordingly, the project aims to incorporate Fe(III) in CNDs through an one-pot, microwave-assisted synthesis to fabricate suitable Fe-based nano-contrast agent. Fe-doped CNDs (FeCNDs) were synthetized in a bottom-up approach from β-alanine, a chelating agent and a Fe(III) precursor. The crude was obtained after centrifugation and dialysis, and final FeCNDs were purified from other molecular-like side products through size exclusion chromatography. Pure FeCNDs were obtained as negatively charged particles, with spherical-like shape and a size distribution ranging between 1-10 nm, as measured by AFM. The presence of iron was confirmed by ICP-MS and XPS measurements. Fluorescence spectroscopy evidenced an excitation wavelength dependent emission, confirming the formation of the carbon nanoparticles. Moreover, FeCNDs displayed promising results as T_1 contrast agents. These results pave the way for the use of FeCNDs as an alternative to Gd-based contrast agents.

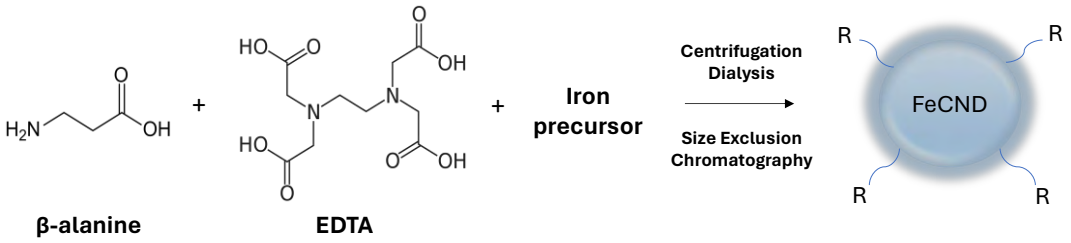

Figure 1. Schematic synthetic pathway for the formation of FeCNDs as nano-contrast agent.

Keywords: Carbon Nanodots, MRI, Iron.

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Selenium, Gadolinium (III)-doped carbon dots as multimodal platforms for medical imaging and therapy

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Carbon dots (CDs) are zero-dimensional quasi-spherical carbon nanomaterials smaller than 10 nm. Due to their inherent properties including tunable photoluminescence, good water solubility, and especially their low cytotoxicity, they have been studied for many applications in biomedicine [1]. Furthermore, the incorporation of heteroatoms in CDs has also been exploited to add functionalities to the material. Selenium plays important roles in selenoproteins, such as glutathione peroxidase (GPx), which are essential for regulating the redox homeostasis in the body [2]. Meanwhile, gadolinium has been used in Magnetic Resonance Imaging (MRI) contrast agents owing to its paramagnetic effect. However, due to the toxicity given by Gd(III)-leakage, the incorporation of these ions into more stable environments is to be pursued [3]. Considering all this, the present work aims to develop selenium, gadolinium (III)-doped CDs (Se,Gd(III) CDs), a platform possessing a dual nature as an MRI contrast agent and an antioxidant. The synthesis of Se,Gd(III) CDs has been performed with a bottom-up approach, through microwave-assisted hydrothermal reactions. Se,Gd(III) CDs, having a mean size of 5 nm, were determined to contain 1.5% and 3% w/w of selenium and gadolinium, respectively. The antioxidant properties of the material have been verified with colorimetric assays. Moreover, good magnetic properties have been demonstrated by the high r_1 and r_2 values obtained with relaxation measurements, suggesting its possible use as a positive contrast agent. Finally, the stability of the material has been established for over 15 days. The successful development of Se,Gd(III) CDs could confer theranostic benefits in inflammation-based and reactive oxygen species (ROS)-linked diseases.

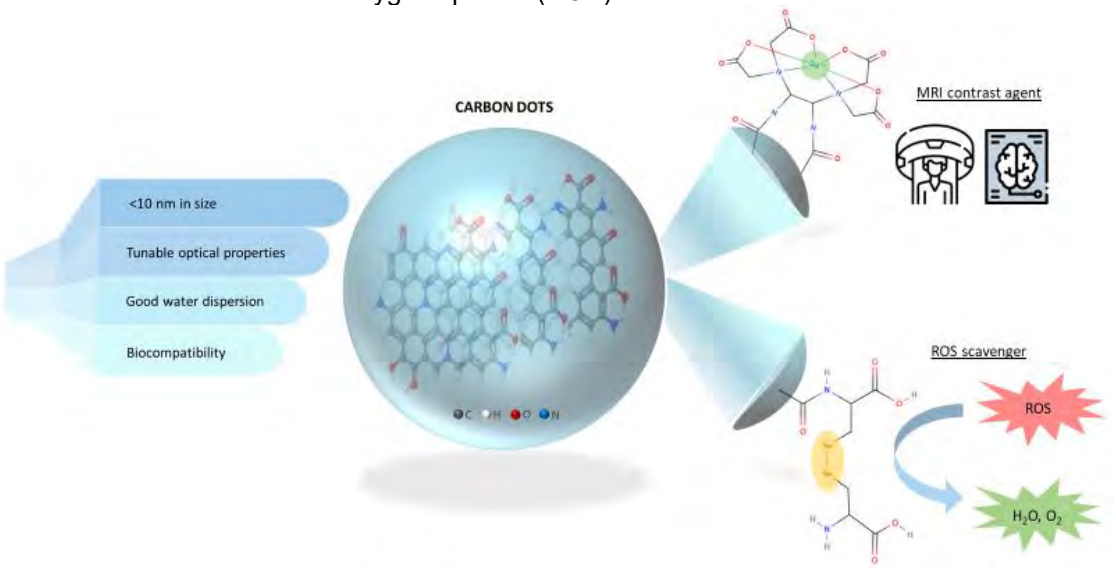

Figure 1. Se, Gd (III)-containing carbon dots as a potential dual-mode platform for biomedical applications.

Keywords: carbon dots, selenium, gadolinium, MRI, antioxidant.

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3D-printed dynamic alveolar models

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Our aim is to develop a dynamic 3D-printed *in vitro* lung model focused on the air-blood interface including human derived cells, to study the underlying mechanisms of different lung diseases and to be used as drug screening platforms (1). This is achieved by digital light process (DLP), printing a net-like structure resembling the human alveolar cavities (2), and by employing hybrid functional 4D biomaterials that provide different functionalities, such as cyclic expansion-contraction behavior (vinyl caprolactam, VCL, and gold nanorods, AuNRs), flexibility and photopolymerizability (polyethylene glycol diacrylate, PEGDA, and lithium phenyl-2,4,6-trimethylbenzoylphosphinate, LAP) and charges to improve cell adhesion ([2-(Methacryloyloxy)ethyl]trimethylammonium chloride, DMC). The cyclic dynamics, aimed to imitate the breathing of the native lung, is achieved by irradiating the biomaterial with pulses of a near-infrared (NIR) laser, triggering a localized heating of AuNRs. The temperature of the material transitions between below and above the Lower Critical Solution Temperature (LCST) of the VCL, generating the expansion-contraction movements (3). The swelling, LCST and mechanical properties of the constructs vary depending on the concentrations and proportions of the monomers added. This allows us to tune the materials to resemble the properties of healthy or diseased tissues. Moreover, the hybrid polymer is biocompatible with the cell lines present in the alveolar region (endothelial and lung epithelial cells). Additionally, a bioink derived from decellularized lung extracellular matrix (lung dECM) and including lung epithelial cells and fibroblasts will be combined with the honeycomb scaffold. A decellularization protocol for porcine lung has been implemented, where the cells are successfully removed while maintaining a similar ECM structure of the native lung.

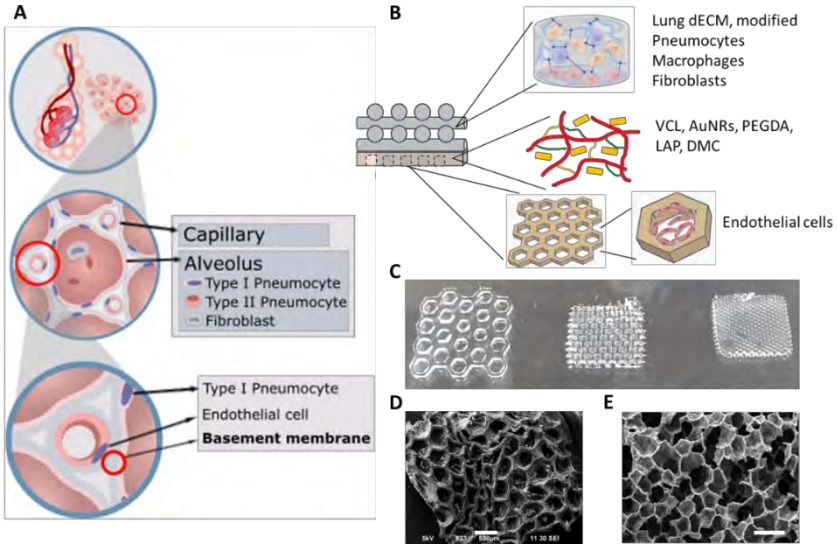

Figure 1. Initial approach for DLP printing a model of the air-blood barrier of the lung. (**A**) Illustration showing the organization of the alveoli. (**B**) Schematic representation of the different layers included in the model and their composition. (**C**) Photograph of DLP-printed scaffolds with a honeycomb shape. The designs were printed starting from bigger to smaller scales, aiming for the human alveolar cavity size. (**D-E**) SEM micrographs of the lyophilized scaffold (smallest scale printed) (**D**) and human lung (from (2)) (**E**), showing a similar structure and size (both scale bars: 500 µm).

Keywords: hybrid biomaterials, alveoli, 3D printing, stimuli-responsive, DLP.

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Development of first-in-class PET tracer for T-Type Calcium Channels (TTCCs)

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T-type calcium channels (TTCCs) are involved in fundamental physiological processes such as neuronal excitability, nociception and heartbeat. Upregulation of TTCCs have been linked to severe pathological conditions such as absence epilepsy, neuropathic pain and hypertension.[1–4]
Despite their paramount role in the development of these diseases, imaging tools for in vivo studies are still lacking. Herein, we focused on the development of new PET probes to elucidate the role of TTCCs in diseases.
The probe design was based on the scaffold of a well-known TTCCs ligand, Z944, and involved the synthesis of a precursor (1) followed by late-stage fluorination with ^{18}F to obtain the first TTCCs radiotracer (Figure 1).[5]
Z944 is a drug candidate that reached phase II clinical trial, which has been shown to bind TTCCs with excellent selectivity and high potency (IC_{50}: 50 to 160 nM, depending on the Ca_v3 isoform) and has proven metabolic stability.[1,6,7]
In this work, we report the synthesis of the precursor (Compound 1). The desired compound (1) was achieved through a multi-step convergent synthesis, involving peptide coupling, deprotection steps and nucleophilic substitution reactions. The final compound was characterized via ^{1}H and ^{13}C NMR as well as via HR-MS.
This represents the perfect precursor for the ^{18}F-radiolabeling, thus paving the way to radiolabelling and test of the compound in PET imaging. Future biological applications include diagnosis of cardiovascular diseases (CVDs), that today still represent one of the major causes of deaths worldwide.[8]

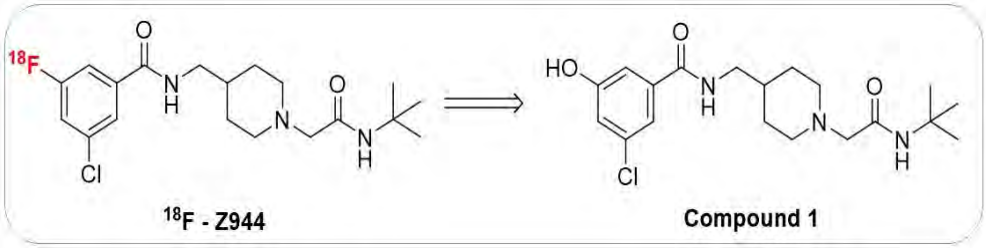

Figure 1. ^{18}F radiolabelling of the first PET tracer precursor for TTCCs.

Keywords: T-type calcium channels, heart, cardiovascular diseases, positron emission tomography, radiotracer.

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New metabolic modulators based on organometallic Iron intracellular catalysts

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Recent work in the field of chemical biology has explored the use of transition metal catalysts inside cells. Treatments based on catalytic drugs are exciting, as small doses can help to transform large numbers of substrate molecules to achieve the desired activity.[1-] As such, we intend to develop new iron catalysts capable to reduce ketones inside cells through a transfer hydrogenation (TH) reaction.[2]

Ketones, are key components of different metabolic pathways that are involved from the production of energy (ATP) to the synthesis of biomolecules (i.e., glucose, steroids, amino acids, etc.). Therefore, targeting ketones such as pyruvate or acetoacetyl-CoA inside cells might be a good way to treat diseases such as diabetes or hypercholesterolemia (respectively) by modulating metabolic pathways related to them. However, stability and toxicity are current issues that still need to be addressed before any metal catalyst can be used as clinical or biotechnological agents. Degradation decreases catalytic efficiency, and lead to unwanted toxic effects upon release of free metal ions.

Based on our previous work,[3] we will re-design the Knolker's catalyst to include halogen tags (such as F, Br or I) in different parts of their structure. This will allow to follow the biological behavior and stability of the catalysts using a combination of PET, MRI, and synchrotron-based X-ray techniques. Such information will help developing improved non-toxic and stable derivatives for the *in cellulo* reduction of ketones, and advancing towards therapeutic agents for the control metabolic disorders.

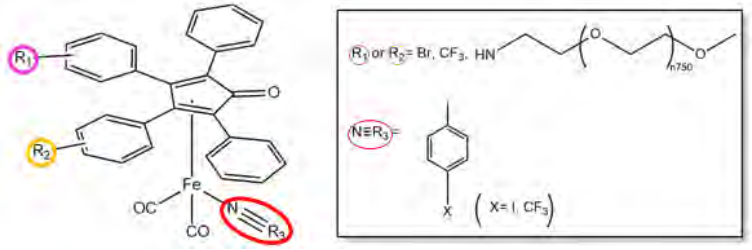

Figure 1 *Knolker's catalyst with the intended modifications with spots marked with purple, yellow and red circles.*

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