AMYC BIOMED 2024

Autumn Meeting for Young Chemists in Biomedical Sciences

5th Edition

Rome 23rd-25th September 2024

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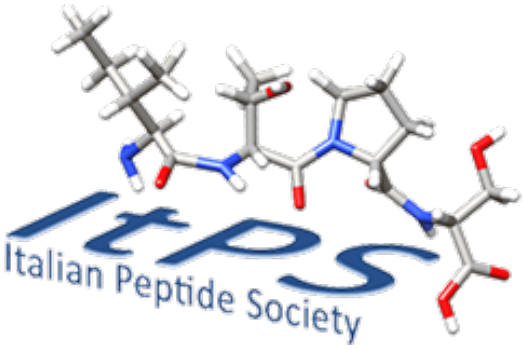

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PLENARY SPEAKERS

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Design of multifunctional conjugates for the targeting of non-coding RNAs: anticancer and antimicrobial applications

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Team Targeting of Nucleic Acids https://icn.univ-cotedazur.fr/tna

RNA is one of the most intriguing and promising biological targets for the discovery of innovative drugs in a large number of pathologies and various biologically relevant RNAs that could serve as drug targets have already been identified (1). Among the most important ones, it is worth to mention prokaryotic ribosomal RNA which is the target of a number of currently employed antibiotics, viral RNAs such as TAR, RRE and DIS RNA of HIV-1 or oncogenic microRNAs that are tightly involved in the development and progression of various cancers. However, difficulties in the rational design of strong and specific small-molecule ligands renders this kind of molecules relatively rare. In this presentation, we will show our recent results about the structure-based design of new RNA ligands targeting oncogenic RNAs that led us to the identification of new compounds bearing a promising biological activity but also to a better understanding of the formed interactions toward the design of optimized compounds (2). In parallel to the design of bioactive compounds, we also perform the screening of chemical library thus increasing the available chemical tools for the development of efficient and specific RNA binders for a wide number of therapeutic applications (3). We will finally show the validation of a new antibacterial target and the design of original compounds bearing potential antimicrobial activity against resistant bacterial strains.

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HYDROGEL-PLASMONIC NANOCOMPOSITES: A VERSATILE PLATFORM FOR OPTICAL (BIO)SENSING

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Novel hybrid nanocomposites, based on polymer hydrogels and metal nanoparticles, are proposed as a multifunctional platform. The innovative fabrication technique assures high reproducibility, large scalability, and simplicity of a one step strategy producing a nanocomposite material whose optical properties can be easily predicted within the hybrid nanoparticles framework. The mechanical properties of hydrogels with different molecular weights easily tune the optical features of the hybrid nanocomposites that are exploited to obtain devices with different transduction mechanisms, exhibiting excellent sensing performances in the selective and sensitive detection of a model target molecule. The presented devices find direct applications in wearable biosensors, food and environmental monitoring.

AMYC BIOMED 2024

Organisms-on-a-chip

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Abstract. The progression of fibrosis is frequently related to a failed healing process, and it may affect many tissues and organs causing severe consequences including post-infarct heart insufficiency, post-injury limb paralysis, cirrhosis, nephropathy, retinopathy, failure of implanted devices and even resistance to chemotherapy in solid tumours. Experimental models, both in vitro and in vivo, are widely used for studies of basic pathophysiology, and for pre-clinical testing of pharmacological therapies counteracting fibrosis. However, conventional models are not able to realistically reproduce the revascularization aspect of the inflammatory reaction that leads to the generation of fibrotic tissue. In this talk, I will present the most advanced frontier in this sector, represented by the new concept of an "organism-on-a-chip". This is a hybrid model, in which an organ-on-a-chip is implanted sub-cute in a living organism, such as a mouse or an embryonated avian egg, thus eliciting a foreign-body reaction with the formation of a fibrotic microenvironment. In an organism-on-a-chip, the fibrotic reaction can be guided in terms of extra-cellular stiffness and vascularity, using microscopic scaffolds incorporated in the implanted chip. The fibrotic microenvironment can then be imaged longitudinally, in high resolution, with the added advantage of significantly reducing animal sacrifice. Organisms-on-chip have the potential to become the most ethical and standardizable testing platforms to boost the clinical translation of new therapeutic products in all the medical fields interested by fibrosis.

1 Models of fibrotic progression: state-of-the-art

Great research efforts are currently devoted to the development of new modelling tools for understanding fibrotic progression in many diseases and in performing preclinical tests on antifibrotic drugs. Fibrosis can adversely affect wound healing of the heart and spinal cord after an acute damage, entire organs including the liver and the kidney, encapsulation of an implanted biomaterial, and can cause drug resistance in a solid tumour. Realism of the study models in these fields is directly proportional to the potential of clinical translation of the results. Even in animal models of fibrotic progression developed from human cells, anti-fibrotic agents have a low effectiveness resulting from their poor penetration into fibrotic masses and suboptimal pharmacokinetics.

1.1 Humanised organoids and assays *in vitro* for fibrosis research

To overcome these limitations, new “humanized” cell models are being developed *in vitro*. Cell spheroids and organoids from human cells are scarcely reproducible and cannot include interactions with the tumour extra-cellular matrix. Microfluidic-based platforms can instead recreate on micro tissues *in vitro* complex functional aspects of the fibrotic micro environment. For example, a breast tumour-on-chip may reproduce differential fibroblast activation modulating extra-cellular matrix diffusivity causing treatment resistance. The sector of organ-on-chips has evolved at a level that these platforms are now starting to be integrated into the drug development pipeline, in partial replacement of animal studies. However, even the most advanced organ-on-chip platform lacks a functional microvascular network. At the state of the art, microvascular networks generated *in vitro* aren’t stable enough in time, in terms of alignment, hierarchy and permeability, to maintain physiological flows in the vessels’ lumens. This limits the realism of these platforms in reproducing fibrotic progression, because it is precisely signalling between endothelial cells, fibroblasts and macrophages that gives rise to the real organ stiffening behaviour.

1.2 Models of fibrotic progression with intravital imaging *in vivo*

Alternatively, an evolving metastasis, or the fibrotic encapsulation of a polymeric scaffold, can be imaged *in vivo* with two-photon intravital microscopy, through a permanent imaging window surgically implanted in a rodent. For example, observation of a human breast cancer xenograft, around 4 mm in diameter, through a mammary imaging window allowed to monitor tissue revascularisation and oxygenation at various time points of tumour progression. However, in this model optical distortions limited the accuracy of parameter quantifications on intravital two-photon images, due to significant changes in the tissue refractive index with light penetration depth. Most importantly, after 11 days of observation, cancer cells were necrotic in the tumour core and revascularisation occurred only on the tumour periphery thus recapitulating the very initial phase of inflammation that gives rise to progressive fibrotic stiffening of a tumour. As a matter of fact, tumour stiffening takes several months to develop in adult animals, while longitudinal intravital imaging studies can hardly exceed one month. Long-term imaging acquisitions on the same animal must in fact be progressively shortened, due to increasing suffering of the animal by repeated and prolonged (>1h) inhalation anaesthesia.

2 Models of fibrotic progression in embryonated avian eggs

The development of tissue is significantly faster in embryonic organisms. Also, embryos can be long-term two-photon imaged intravitally without compromising their development. The embryonic organism most widely used in pharmacological research is the embryonated chicken egg, not considered a living animal by national legislations worldwide. A chick embryo that has not reached the 14th embryonic development day does not experience pain and can be used for experimentation without any ethical restrictions or prior protocol approval, nor does it require an animal facility. The avian embryo is widely used in studies with human cells because it's immune-tolerant, i.e. its adaptive immune response based on T-cells and antibodies is immature and does not reject xenogeneic material like human cancer cells. Instead, the avian innate immune response based on macrophages is active throughout embryonic development, making an avian embryo model potentially highly predictive, although not entirely human, in replicating an innate response like fibrosis. This experimental model allows to measure *in vivo* the effect of therapeutic agents injected in the embryonic circulation, on pharmacokinetics, toxicity, pro/anti-angiogenicity, tissue repair and wound healing induced on the embryo and/or, most interestingly, on a breathing structure in contact with the egg shell, called chorioallantoic membrane (CAM). Transparent eggshells may improve optical accessibility of the CAM to intravital microscopy.

2.1. Measurement of a foreign-body fibrotic reaction in an organism-on-a-chip

We have recently developed and patented an innovative platform, consisting of a micro-patterned imaging window implantable *in vivo* [1]. To validate this window in an *in vivo* environment, we implanted the window in chicken embryos extracted from the egg shell and maintained in culture *ex ovo* (Fig. 1). Inside these micro scaffolds, we measured by label-free two-photon microscopy the infiltration of cells and micro vessels, and the collagen content at 4 days after implantation. Then, we euthanized and fixed the embryos with a paraformaldehyde solution. We dissected embryos to perform immunofluorescence treatments focused on immune response labelling to confirm all the data collected by two-photon imaging. We performed single-photon multi-stack acquisitions of the immune-labelled infiltrated scaffolds comparing linear and nonlinear microscopy in terms of cellular density, blood vessels infiltration and collagen-I content. Confocal and two-photon observations at implantation sites demonstrated massive cell infiltration inside the micro scaffolds, with cell density triplicated in 24 hours, and a capillary density almost six-fold greater in the micro scaffolds than in surrounding control tissue. Second harmonic generation signal showed a highly oriented layer of collagen-I inside the micro scaffolds [2].

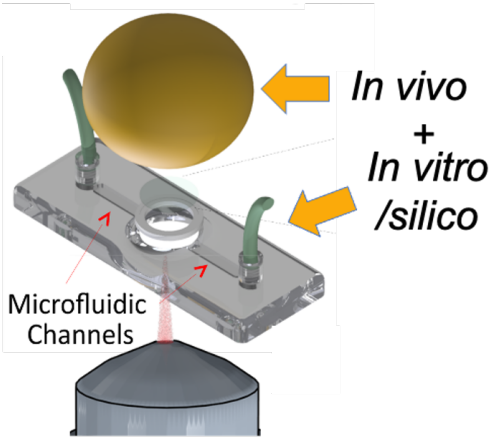

Fig. 1. The emerging concept of an organism-on-a-chip. A microfluidic organ-on-a-chip is implanted in a living organism and imaged intravitally in high resolution.

We also measured fibrotic reactions in non-cellularised micro scaffolds implanted subcutaneously in adult mice for 7 weeks. Our preliminary data show that implantation of micro scaffolds does elicit a foreign-body fibrotic reaction, dependent on the scaffold micro architecture and well quantifiable in real time *in vivo*.

In conclusion, our new organism-on-a-chip platform has the potential to shorten the approval process for drugs, using a combined efficacy/safety approach that lowers the risks.

This project is funded by the European Research Council (ERC, project BEACONSANDEGG, G.A. 101053122) and by the European Commission (EU, FET-OPEN project IN2SIGHT, G.A. 964481).

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INVITED SPEAKERS

Peptide-based recognition of viruses by natural killer cells

Hammer Quirin

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Natural killer (NK) cells are innate immune cells that contribute to host defense against virus infections. The functions of NK cells are tightly regulated by a balance of activating and inhibitory receptors. The inhibitory receptor NKG2A binds to complexes of human leukocyte antigen E (HLA-E) and peptides on the surface of healthy cells, preventing autoimmunity through inhibitory signals. Accordingly, loss of HLA-E/peptide complexes sensitizes target cells due to reduced inhibition. Some viruses such as cytomegalovirus exploit this inhibitory axis for immune evasion by providing a viral peptide that is presented by HLA-E and instills recognition of self to NK cells. In this context, we study the sequence and structural features of viral peptides presented by HLA-E and how this relates to immune responses of NK cells.

For instance, we show that a peptide encoded by the Non-structural protein 13 (Nsp13) of SARS-CoV-2 is efficiently presented by HLA-E. Despite having an atypical sequence, the viral $Nsp13_{232\text{-}240}$ peptide is appropriately positioned in the peptide binding groove, as supported by molecular dynamics simulations as well as crystal structure analyses. In contrast to canonical self-peptides, presentation of the viral $Nsp13_{232\text{-}240}$ peptide prevents binding of HLA-E to the inhibitory receptor NKG2A. This absence of binding to the receptor results not in immune evasion but rather renders target cells susceptible to NK cell attack due to reduced inhibition. In line with these observations, NKG2A-expressing NK cells proficiently respond to SARS-CoV-2-infected lung epithelial cells and are activated in patients with COVID-19. Finally, we observe that emerging variants of SARS-CoV-2 have acquired a mutation within a key position of the $Nsp13_{232\text{-}240}$ peptide that impairs its presentation by HLA-E and is associated with enhanced viral fitness.

Collectively, these data suggest that a viral peptide presented by HLA-E abrogates inhibition of $NKG2A^+$ NK cells, thereby enabling the recognition of infected cells. Moreover, our findings imply that this recognition mode exerts immunological pressure on the virus, highlighting an intriguing host-pathogen interaction.

Motile DNA-based nanorobots for nanomedicine

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In the last years, researchers turned to the remarkable multifunctionality of biological systems as a source of inspiration for engineering "intelligent" artificial active nanosystems (nanorobots) capable of performing complex tasks at the nanoscale, including active motion by biocatalysis. One of the most sought-after applications of such devices is nanomedicine, where our group has pioneered studies on the use of active nanosystems to improve the delivery of anti-cancer drugs, both *in vitro*[1] and *in vivo*[2,3]. A plethora of different materials such as polymers, lipids, silica, gold or platinum have been employed to construct biologically inspired motile micro- and nanosystems, achieving precise and predictable control over the assembly of structural and functional building blocks remains a challenge. DNA nanotechnology offers a promising avenue for constructing increasingly complex molecular nanomachines by utilizing synthetic DNA strands that can self-assemble into highly defined and predictable 2D and 3D nanostructures.[4] Moreover, this approach allows for the precise and programmable molecular patterning of functional moieties, including fluorescence labels, cell recognition elements, and enzymes.

Here, we explore the use of synthetic DNA strands to engineer hybrid catalytic nanoswimmers highly integrated functional capabilities, including environment sensing, [5] controlled motility[6] and cellular interactions.

We believe that incorporating DNA nanotechnology as a tool for engineering nanorobots holds tremendous potential for unlocking new emergent behaviors and functionalities that can be applied in nanomedicine, including improved transport of medical agents across biological barriers, enhanced tissue penetration and programmable cellular interactions.

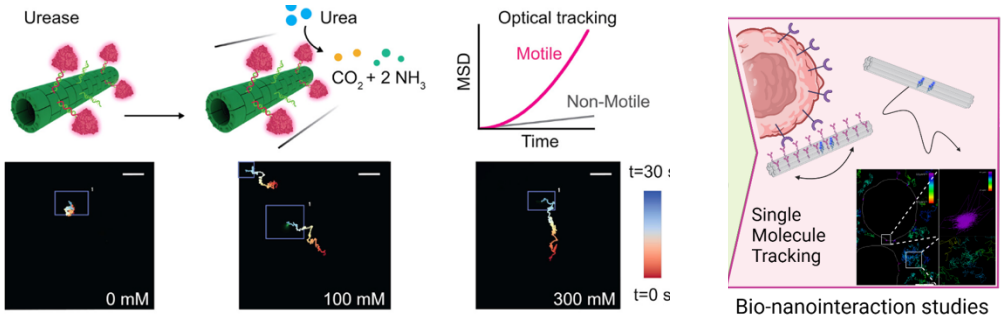

Figure 1. Examples of DNA-based nanorobots motility and cell interaction studies.

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Non-persistent nano-architectures and alternative biomodels in translational cancer research

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Keywords: oncology, nanotoxicology, noble metals, alternative biomodels

During 90's, nanotechnology has produced a first revolution in the clinical treatment of neoplasms through the release of Caelyx®/Doxil®, a liposome formulation of doxorubicin. Beyond the drug delivery enhancement associated to nanomaterials, noble metals are essential for the development of combined/enhanced non-invasive cancer management approaches.[1] Actually, because of the lack of a rationale design and adequate biodistribution and safety data, noble metal nanomaterials are still not in the market.[2] In this scenario, elucidate the fate of metal nanomaterials once administered is critical to compose safe-by-design nano-therapeutics.[2]

Here, an introduction on nanotechnology applied to oncology is provided together with the next exciting perspectives and challenges to address in translational cancer research. Moreover, the bio/nano-interactions, biodistribution, and clearance profiles of hybrid nano-architectures (NAs) are presented and the impact on the biokinetics of the chemical nature of noble metals and of the administration strategy discussed.[3,4] The last achievements from the preclinical application of NAs in the treatment of primary tumours, metastasis, and damaged skin are reported along with the pivotal role of alternative biomodels in the oncological research workflow.[5–7]

The research leading to these results has received funding from AIRC under MFAG 2017 – ID 19852 project – P.I. Voliani Valerio.

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ORAL COMMUNICATIONS

From safety to sustainability along the tomato supply chain

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Keywords: tomato; metabolomics; food; sustainability

Tomatoes and tomato-based food are worldwide diffused foodstuffs, whose nutritional importance is related to their contents of many antioxidant compounds, like carotenoids, polyphenols, and vitamins [1-3]. However, tomatoes are a food category extremely exposed to safety risks that can be related to the presence of chemical residuals, like pesticides [4], and microbial contaminants, including bacteria and fungi, as well as characterized by a significant amount of solid waste, including vascular tissue, seeds and peels. [1,5-6]. In this context, the present study, carried out in the frame of the Agritech consortium [7] stemming from the National Recovery and Resilience Plan (NRRP), is aimed at the metabolomic profile and possible identification of new and (re)-emerging hazards of an old tomato fruit ecotype, i.e., "Pomodoro Riccio". This is a cultivar particularly suited to grow up on clay soils, with a low demand for water, that could ultimately mean a lower intake of contaminants. In fact, it has been proven that water is the primary source of heavy metals and pesticide residues [4], thus, this old cultivar has the potential to enhance the quality of tomatoes that emerges as a matter of priority for customer's safety. Pomodoro Riccio samples (fresh tomatoes, tomato paste and waste) harvested in two different years were provided by "La Sbecciatrice" company (Caserta, Campania). The hydroalcoholic and organic Bligh-Dyer extracts have then been subjected to the multimethodological analytical protocol comprising untargeted (NMR, FT-ICR MS) and targeted (HPLC-DAD, HPLC MS/MS) methodologies. In particular, the high sensitivity and mass accuracy typically achieved with FT-ICR MS implies that elemental formulas of many metabolites and harmful compounds present in trace amounts, like pesticides, agrochemical derivatives and metals, can be determined. Overall, an extensive chemical and metabolomic profile was collected to monitor the safety and quality of the products, as well as the environmental impact and sustainability of the entire agricultural production process.

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Lipid self-assembling nanoparticles for the development of novel mRNA-based vaccines

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Keywords : Vaccines, RNA, Lipid nanoparticles, Neurodegeneration.

Messenger RNA (mRNA) has broad therapeutic potential for a variety of applications, including neurodegenerative diseases such as, brain cancer and Alzheimer's disease [1;4]. However, the full development of therapeutic strategies based on the use of mRNA is hampered by its disadvantageous biopharmaceutical profile, requiring the use of vectors to ensure effective mRNA delivery [1]. Lipid nanoparticles (LNPs) have been approved for clinical use to deliver oligonucleotides or mRNA but have some drawbacks such as poor physical stability, which requires storage at temperatures below -20 °C [3]. Our research group developed lipid self-assembling nanoparticle (SANP) as an alternative platform for RNA delivery to overcome these issues. Lipid SANP formulations have shown remarkable biocompatibility, high RNA encapsulation efficiency, and enhanced intracellular release. Furthermore, SANP have been designed to be prepared at room temperature immediately before use by simple mixing three components, a calcium phosphate dispersion, RNA, and cationic liposomes (**figure 1**). By using this approach, the RNA can be stored in a lyophilized form, which ensures greater stability against degradation compared to freezing. To achieve mRNA-loaded SANPs with hydrodynamic diameters below 200 nm, a homogeneous nanoparticle population (PDI < 0.2), high mRNA encapsulation efficiency, and good stability against aggregation in serum, various SANPs formulations were developed by varying the lipid composition and ratios of lipids, as well as the ratio of lipids to mRNA. These optimized SANP loaded with mRNA encoding a green fluorescent protein showed efficient *in vitro* transfection on HELA cells. Furthermore, SANP encapsulating a mRNA encoding alkaline phosphatase yielded protein production upon intramuscular (i.m.) or intravenous (i.v.) administration in BALB/c mice. Finally, SANP loaded with mRNA encoding the spike protein of COVID-19, herein used as a model antigen, induced an immune response *in vivo* upon i.v. or i.m. injection with no associated renal and hepatic toxicity. These results make SANP formulations promising for mRNA-based vaccines, allowing to overcome one of the main barriers to the distribution of current mRNA vaccines [3].

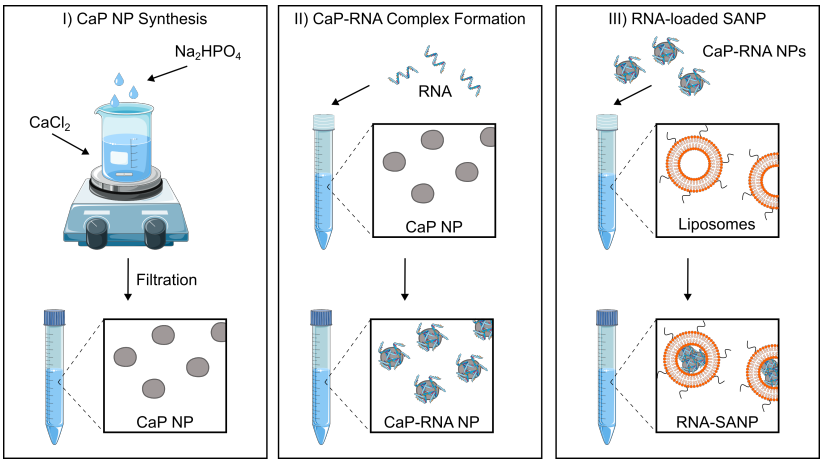

Figure 1. Preparation of SANPs: synthesis of NP CaP (I), formation of the CaP-RNA complex (II) and addition of CaP-RNA complexes to liposomes (III).

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AMYC BIOMED 2024

Laser scattering of gold nanoparticles-decorated Ti_3C_2 MXene for enhanced optical biosensing

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Keywords: MXenes, gold nanoparticles (AuNPs), biosensors, Sensor phenomena, laser-based sensors

Laser based optical sensors have demonstrated outstanding performance for numerous analytical applications. Despite their sensitivity, require difficult substrate preparation, longer data acquisition times, and complex operations. Here we present an optical sensor based on photodetection of laser light scattered by the flow of nano-microparticles and nanocomposites. In addition, to interpret output data and reduced measurement duration, we developed a peak analyzer algorithm which analyse the intensity distribution and the number of peaks, thus extracting the concentration data **(Fig. a)**. MXenes offer enhanced solubility in aqueous-based environments due to their negative zeta potential, making them useful for biosensing via optical transduction. To create nanocomposites, electrostatic self-assembly approach was employed to create AuNPs@$Ti_3C_2T_x$ nanocomposite (~1 µm size). For biosensing, AuNPs@$Ti_3C_2T_x$ were functionalized with anti-human I_gG (f-AuNPs@$Ti_3C_2T_x$) via Photochemical Immobilization Technique (PIT). When f-AuNPs@$Ti_3C_2T_x$ encounters the analyte (human IgG), aggregation occurs following antibody-antigen interaction and forming clusters of two or more of these nanocomposites. As the analyte concentration increased, more aggregation happened, resulting in the formation of larger clusters and a decrease in the number of peaks **(Fig. a)**. Let N_i represent the initial concentration of f-AuNPs@$Ti_3C_2T_x$ nanocomposites per mL. If an aggregation occurs, final concentration or number of particles becomes $N_f = N_i/2$ (and for n aggregations; $N_f = N_i/n$). Therefore, we used N_f as a sensing parameter to investigate how the analyte induced f-AuNPs@$Ti_3C_2T_x$ aggregation. For detection experiments, the concentration of f-AuNPs@$Ti_3C_2T_x$ was set at 1 for absorbance's value on the plasmonic gold peak ≈ 530 nm. An aggregation experiment was performed with f-AuNPs@$Ti_3C_2T_x$ nanocomposites under different analyte concentrations such as 0.1, 0.5, 1, 2.5, 5 ng/mL. The experiment lasted 48 s with a flow rate set at 12.5 mL/min. With no analyte in the colloidal solution mean no aggregation and peak intensity was higher. Upon adding analyte, aggregation occurs and number of peaks started decreasing until 0.5 ng/mL **(Fig. b)**. Beyond this value, aggregation stopped, and the detected peaks rises again (emergence of Hook effect). The discussed laser scattering sensor is sensitive enough to detect small fluctuations in the particle number caused by antibody-antigen interactions. Here, AuNPs@$Ti_3C_2T_x$ show higher sensitivity and lower LOD than NPs alone. This improvement can be attributed to the extensive surface of MXene flakes and terminations, which anchor numerous AuNPs. Combining 2D materials with a fluidic system can lead to rapid, sensitive, and cost-effective detection of various analytes, enabling high-throughput analysis, miniaturization, and automation.

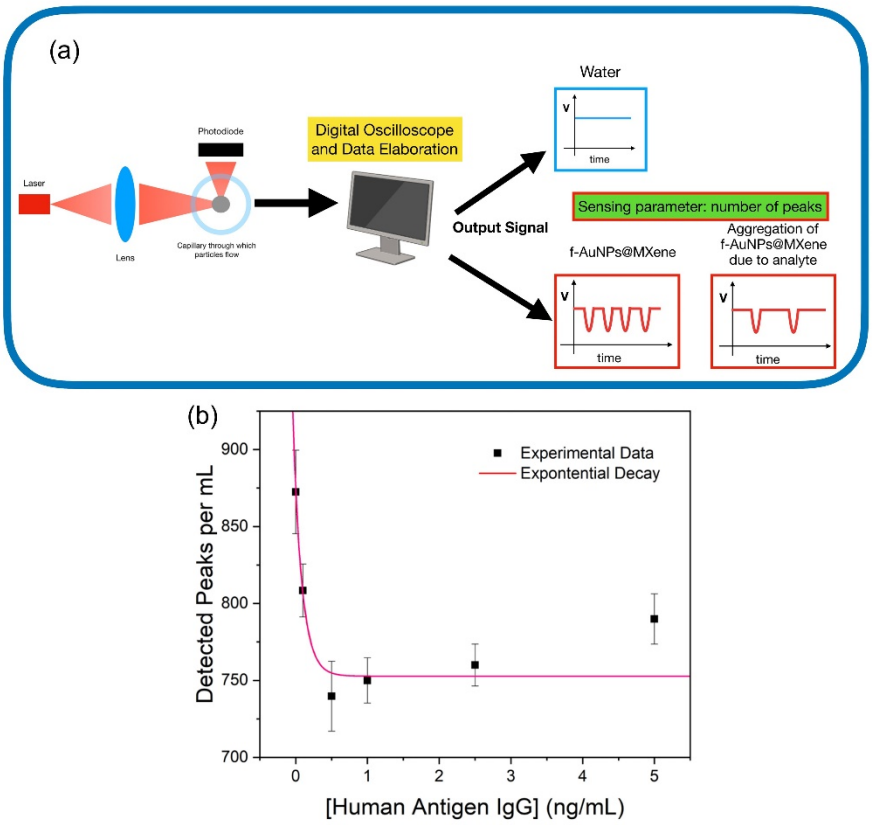

Figure: (a) Schematics of sensing assembly, data acquisition and sensing principle. (b) Number of peaks/mL recorded coming from the aggregation of f-AuNPs@$Ti_3C_2T_x$ at various antigen concentrations. Following antibody-antigen interactions, aggregation increases, this reducing the number of particles. Error bars from eight separate data collections were evaluated.

Reference:

Francesco Pisani, Ayesha Zaheer, **Zaheer Ud Din Babar**, Raffaele Velotta, Carmine Granata, Fabrizio Tessicini, Bartolomeo Della Ventura, Vincenzo Iannotti, "Unveiling Biosensing Innovations: Laser Scattering Sensor and Gold Nanoparticles-Decorated Ti_3C_2 MXene Composite for Enhanced Biosensing Applications," in IEEE Sensors Letters, vol. 8, no. 7, pp. 1-4, July 2024, Art no. 1501204, doi: 10.1109/LSENS.2024.3407530.

How R3τ drives metal-mediated oxidative stress in AD

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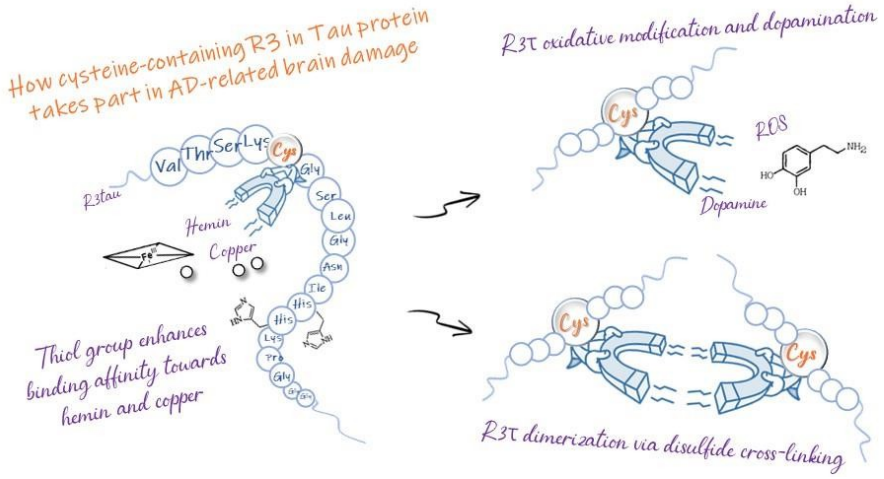
Keywords: Alzheimer's disease – Oxidative stress – Post-translational modification – Metal dyshomeostasis



Tau is a microtubule-associated protein normally involved in the axonal stabilization but, in pathological conditions, it forms insoluble aggregates, commonly known as neurofibrillary tangles (NTFs).[1]
This protein comprises four pseudorepeats (R1–R4),[2] containing one (R1, R2, R4) or two (R3) His-residues, that potentially act as binding sites for metal ions, i.e. copper and heme iron. Moreover, the presence of two cysteine residues in R2 and R3 might have a crucial role in protein conformational changes and further aggregation, through possible disulfide bond formation, and/or affecting the interaction with redox-active metal ions and the reactivity of the resulting complexes.[3-5]
We, therefore, compare the interaction of copper and hemin with octadeca-R3-peptide (R3C) and with its mutant containing an alanine residue (R3A) to better assess the role of thiol group. In the spectrophotometric titrations of complexes with copper and hemin, a remarkable strong interaction was observed only in the case of R3C.[6] Moreover, copper-R3C and hemin-R3C complexes display a particular oxidative mechanism triggered by the intrinsic reactivity of cysteine residue; this reactivity results in the generation of high levels of disulfide bonds and dopamine- Cys covalent adducts, two events strongly connected to protein misfolding and aggregation associated to AD.

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CRISPR-Powered Real-Time Monitoring of DNA Glycosylase Activity Using Synthetic DNA Translator

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Keywords: CRISPR, Cas12a, DNA repair, Base excision repair, DNA glycosylase

DNA glycosylases are a class of DNA repair enzymes crucial for maintaining genomic stability by identifying and excising damaged nucleotide bases, thereby initiating the base excision repair (BER) pathway. BER plays a vital role in protecting the genome from mutations and associated diseases like cancer. Monitoring DNA glycosylase activity is pivotal for developing targeted therapies and facilitating clinical diagnosis.[1] However, traditional assays for measuring DNA repair activity in biological samples often face challenges such as multi-step procedures, dependency on auxiliary enzymes, or sensitivity issues.

Here, we introduce a CRISPR-Cas12a-powered detection platform tailored for rapid and sensitive measurement of both mono- and bifunctional DNA glycosylases. Our activity-based assay exploits CRISPR-Cas12a-mediated signal amplification triggered by the molecular reconfiguration of a lesion-containing hairpin DNA probe (i.e., DNA Translator). This DNA Translator serves as both the substrate for the DNA repair enzyme and the activator for Cas12a. By utilizing a uracil-containing DNA Translator, we enable real-time monitoring of uracil-DNA glycosylases (UDG), such as human SMUG1 and UNG. UDG excises uracil bases incorporated into the DNA Translator, leading to the generation of apurinic/apyrimidinic (AP) sites and subsequent destabilization of the hairpin structure. Repair-induced hairpin reconfiguration triggers Cas12a nuclease activity and fluorescence signal transduction. We demonstrate that the kinetics of hairpin reconfiguration are specifically induced by UDG activity and can also be temporally regulated by the number of uracil incorporated into the DNA translator. Moreover, by incorporating 8-oxo guanine (8-oxoG) into the hairpin DNA probe, we assess the activity of 8-oxo guanine DNA glycosylases, including Fpg and hOGG1, via a release-upon-repair mechanism induced by the enzyme secondary lyase activity.

Our assay demonstrates precise quantification of cellular DNA glycosylases in real-time, with recovery values ranging between 98-122%. Furthermore, our platform is adaptable for inhibitor screening, making it a versatile tool for clinical diagnosis and drug discovery. This research underscores the expanding role and practical utility of CRISPR technology in diagnostics, offering exciting prospects for enhanced diagnostic capabilities beyond nucleic acid targets.[2]

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Structural and Biological Properties of Novel G4aptemer-drug Conjugates as a Vehicle for 5-fluoro-2′-deoxyuridine

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Keywords: aptamer-drug, 5-fluoro-2′-deoxyuridine, G-quadruplex, T30923 aptamer, cancer therapy

One of the most appealing approaches for cancer treatment is targeted therapy, which is based on the use of drugs able to target cancer cells without affecting normal ones. This strategy lets to overcome the major limitation of conventional chemotherapy, namely the lack of specificity of anticancer drugs, which often leads to severe side effects, decreasing the therapy effectiveness. Delivery of cell-killing substances to tumor cells is one-way targeted drug therapy can work. Aptamer-drug conjugates represent a promising solution to minimize off-target effects, considering aptamers' remarkable selective binding capabilities [1]. Furthermore, recent advances in anticancer agents have highlighted the potential of G4-aptamers in clinical applications [2]. In order to enhance the therapeutic efficacy of the antineoplastic agent 5-fluoro-2′-deoxyuridine (FdU) in various cancer cells, a novel conjugate using the antiproliferative aptamer T30923 (INT) [3] as a drug vehicle was developed. Three derivatives composed of T30923 conjugated with different numbers of FdU units were synthesized, and their structural and biological properties were thoroughly characterized. The collected NMR, CD, and PAGE data strongly suggest that, similar to their unconjugated sequence, the resulting aptamer-drug conjugates showed the ability to fold into a dimeric G4 structure formed by the same G-quadruplexes, characterized by parallel strands, three all-anti-G-tetrads and three one-thymidine propeller loops. Moreover, antiproliferative properties, cellular uptake and endocytosis mechanism have been investigated showing that the coexistence of INT and $(FdU)_n$ not only strengthens the killing effect of free FdU by a synergistic effect, but also enables the conjugates to target and kill cancer cells selectively. In this way, it is possible to minimize side effects, that may arise from the non-specificity or cellular resistance of FdU alone, and also to increase the antiproliferative activity of both compounds.

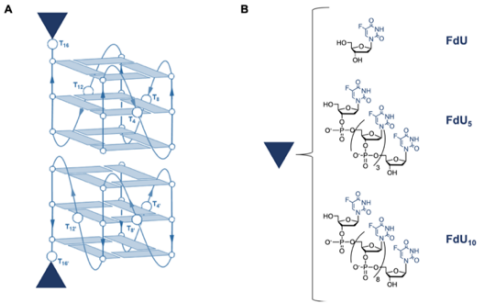

Figure 1. Schematic representation of the G4 structures adopted by the conjugated INT-FdU sequences studied.

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Novel methods on peptide-based cholesterol biosensing

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Keywords: Cholesterol-binding peptide, biosensor, spectroscopy, plasmonic dark field microscopy, poly (lactic acid) membrane

Cholesterol is an essential biological compound in mammalian cells and regarded as a hallmark of worsens diseases as atherosclerosis, Alzheimer or cardiovascular diseases. Therefore, the monitoring of cholesterol and fast determination of its levels in living cells, biosamples as blood or even in food, is a critical point for the analysis and early diagnosis of diverse clinical disorders.[1] Herein it is presented the development and design of novel non-enzymatic cholesterol biosensors based on a selected cholesterol recognition peptide sequence for the analytical detection of free cholesterol molecules in solution by optical spectroscopy and plasmonic and electrochemical measurements. Binding affinity and selectivity of C-pept to cholesterol embedded on model membranes was conducted by fluorescent resonance energy transfer (FRET) obtaining a of $L_{1/2}$ = 24.77 ± 3.3 µM. [2]

An innovative plasmonic cholesterol biosensor was created by merging AVAC technology [3], a dark-field micro-spectrophotometer, for the direct and competitive detection of cholesterol by C-pept.2 The system consisted on the sensing element, C-pept, covalent immobilized on a silica wafer substrate and gold nanoparticles (GNPs) functionalized with PEG-Cholesterol. The read out of counts of the plasmonic GNPs in interaction with C-pept on the surface is based on its optical detection by measuring the light scattering signal of each single GNPs with AVAC. Results indicates the great specificity of the system for the competitive assay with a linear range of the 10 µM -150 µM of cholesterol in solution.

Besides, a biocompatible electrochemical system was created by covalent binding C-pept on the surface of a polylactic acid porous film (PLA).[4] The success of the system was followed and confirmed by electrochemical impedance spectroscopy (EIS) and complementary cyclic voltammetry (CV) with functionalized Screen-printed carbon electrodes (SPCEs) as the transducer substrate and ferrocyanide $Fe(CN)_6^{3-/4-}$ as redox probe. EIS result validated the system and electrochemical cholesterol biosensor with a limit of detection range of 6 µM – 0.04 mM cholesterol in solution. These results lead open alternatives methods for free cholesterol sensing by combining biorecognition motifs with biocompatible polymeric materials which are attracting and desirable for POCT applications, and fast, sensitive and throughput screenings for monitoring and diagnostics.

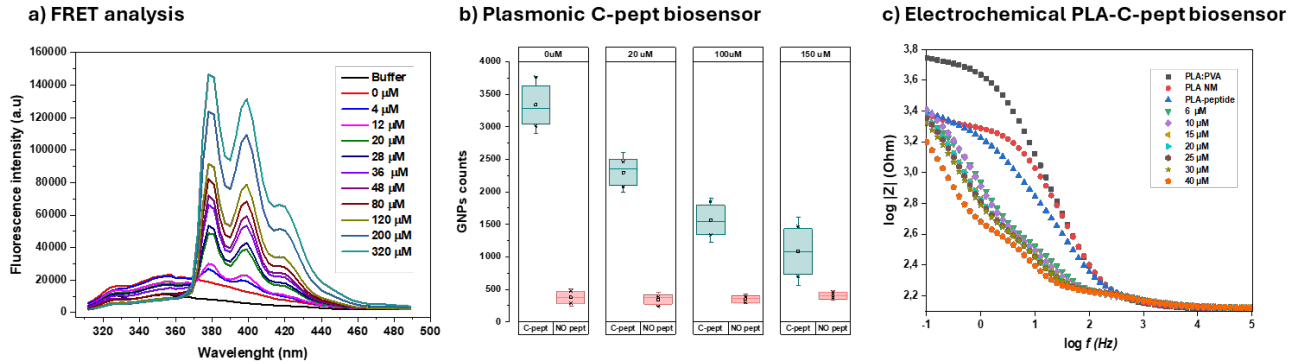

Figure 1.a) Fluorescence emission spectra on FRET assays between C-Pept (Trp) and POPC:Chol(1:0.3)-PyPE 2%liposomes, b) Chart-box plot of the counts of GNPs interacting with the functionalized Si substrate in presence (green box) and in absence (red box) of C-pept as biorecognition element, c) Bode plots of the sequential additions of Chol concentrations into the buffer media containing 5mM (Fe(CN)6-3) redox probe, by employing the PLA-C-pept SPE biosensor.

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Computational simulations of energy transfer in a photosynthetic light-harvesting complex

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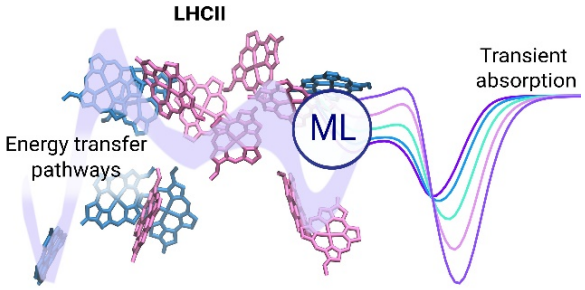
Keywords: photosynthesis, energy transfer, pump-probe spectroscopy, computational chemistry

Photosynthetic organisms have developed efficient mechanisms to extract energy from solar power and learnt to finely tune them to thrive in different environments and adapt to continuously changing weather conditions. The notable sophistication of these processes has inspired a lively area of research which aims at unveiling the molecular processes underpinning of overall photosynthetic performance. Indeed, a deep understanding of natural strategies for light-harvesting would be the key to their own optimization in new practices aimed at, e.g., increasing crop productivity: improving photosynthetic subprocesses for increasing biomass production would eventually contribute to providing an answer to some of the major challenges of our immediate future [1].

The efficiency of natural photosynthesis mostly relies on the optimization of the light-harvesting step. This is accomplished by specialized "antenna" complexes, namely proteins which coordinate high concentrations of pigments [2]. After light absorption, the pigments are involved in a complex dynamics of excitation energy transfer (EET) assisted by the protein scaffold, which funnels the excitation towards a pool of final emitters. Understanding the mechanistic details of such dynamics is still one of the major challenges in the field.

Light-Harvesting Complex II (LHCII) is the largest antenna of higher plants: energy transfer processes taking place inside its aggregate of chlorophylls have been experimentally investigated with spectroscopic techniques [3], but the complexity of the spectral signatures typically prevents the characterization of the transfer pathways at the molecular level. Structure based studies [4,5] have long been trying to shed light on the mechanisms of transfer by employing hybrid approaches, where theoretical models include parameters fitted on experimental data. However, fitting procedures expose to the risk of serious bias in interpretation of experiments, especially when theoretical models carry some important approximations. Conversely, first-principles models, which allow to disentangle experimental data in unbiased ways and naturally provide insights on atomistic details [6], have long been challenged by the large size and complex nature of the system.

In the present contribution, we show that a fully first-principles approach combining molecular dynamics and machine learning can be successfully used to characterize the EET pathways of LHCII [7]. The accuracy of the model and its efficacy in interpreting experiments is tested by simulation of transient absorption spectra. The good agreement between the experimental and simulated spectra validates the proposed mechanisms, this way contributing some new insights to the investigation of natural antennas' working principles.



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Dried blood spot long-chain polyunsaturated fatty acids and Heart Failure disease

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Keywords: dried-blood spot; tandem mass-spectrometry; polyunsaturated fatty acids; inflammation; Heart failure

Heart failure (HF) is a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood.
Dysfunction of cardiac mitochondria is a hallmark of HF and a leading cause of oxidative stress, which in turn causes myocardial tissue damage and inflammation thus contributing to HF progression. The analysis of oxylipins and PUFAs in minimally invasive biological specimens, e.g. oral fluid and dried blood spots (DBSs), can be extremely useful in elucidating their biological activity as well as their potential role as biomarkers in this clinical setting.
Here, a very powerful analytical platform, based on micro-extraction by packed sorbent (MEPS) coupled to liquid chromatography-tandem mass spectrometry (UHPLC-ESI-MS/MS), was fully developed and validated for the targeted profiling of fifty-two oxylipins and four PUFAs in DBSs. The protocol was successfully employed in a pilot study on a cohort of HF and control subjects (n=100) during the cardiopulmonary exercise testing to evaluate metabolic derangements during effort.
We identified key changes in PUFA levels characterizing HF population both at rest and during exercise. HF patients showed generally lower levels of antioxidant omega-3 fatty acids than controls, in line with the "malnutrition-inflammation complex syndrome". Arachidonic acid (AA) significantly ($p < 0.05$) differed between HF patients with preserved, HFpEF, and reduced, HFrEF, ventricular ejection fraction at rest, highlighting a different bioavailability of this circulating omega-6 PUFA. AA levels significantly ($p<0.01$) decreased during the exercise only in HFpEF population, thus suggesting distinctive changes in myocardial uptake and utilization of energy substrates.
These results pave the way for an in-depth molecular understanding of the biological functions of energy substrates and their signalling role, which will be fundamental in the development of novel therapeutic approaches to HF syndrome.

SURFACE FUNCTIONALIZATION OF POLYIMIDE BASED INTRANEURAL DEVICES WITH COVALENTLY BONDED DEXAMETHASONE TO DEVELOP INNOVATIVE DRUG DELIVERY SYSTEMS

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Keywords: Foreign body reaction, polyimide films, surface modifications, drug delivery, anti-inflammatory coating

The inevitable foreign body response (FBR) towards implanted intraneural devices challenges the stability and an active intervention strategy would be desirable to treat inflammation locally. In fact, a fibrotic capsule is formed that physically and electronically isolates the implant from the surrounding tissues, thereby compromising the intimate contact between the sensor and the target cells [1]. This work aims to provide a strategy to covalently link the corticosteroid Dexamethasone (DEX) to the surface of the polyimide (PI)(UPILEX-S®) that is the electronically inert part of the whole implant. This material is particularly suitable due to its properties such as: biocompatibility, hermo-oxidative stability, high mechanical strength, high chemical inertia and heat resistance. The idea is to consider the implant itself as a local delivery system for the drug. In fact, peripheral and systemic administration of DEX has several disadvantages, including multi-organ side effects due to overdosing. The most typical approach is to include the drug on the electroactive surface of the implant. The chemical route consists of a series of modifications on the PI surface in order to attach the DEX. First of all, the imide groups on the surface are hydrolysed in a basic solution [2]. This process generates carboxyl acid functionalities on the activated surface that can be exploited for the subsequent coupling with amines. In particular, an amine rich in hydroxylic groups at one end (Trizma®) is used in this work. These are useful as they provide an ideal platform for the silanization process, which is achieved using triethoxysilylpropyl succinic anhydride (TESPSA), a commercially available silane with a succinic anhydride functionality. The succinic anhydride can act as an anchor site for the insertion of one or two molecules of DEX, through the formation of an ester bond (Figure 1). Hence, the release pathway occurs through the hydrolysis of the covalent bond between DEX and the silane layer. This process can be accelerated by the presence of lytic enzymes (esterases) known to be released during the inflammatory process. FTIR-ATR spectroscopy and SEM-EDX were used to characterize the chemical composition and surface topology of the devices after each step of the functionalization process. In addition, the release kinetics and the whole drug estimation is produced by monitoring the release under neutral pH conditions reproducing. The release profile reaches a plateau after approximately two months, which is expected to mitigate the acute phase of FBR and possibly also reduce chronic adverse reactions. This pioneers the way to construct neural devices capable of attenuating the chronic foreign body response.

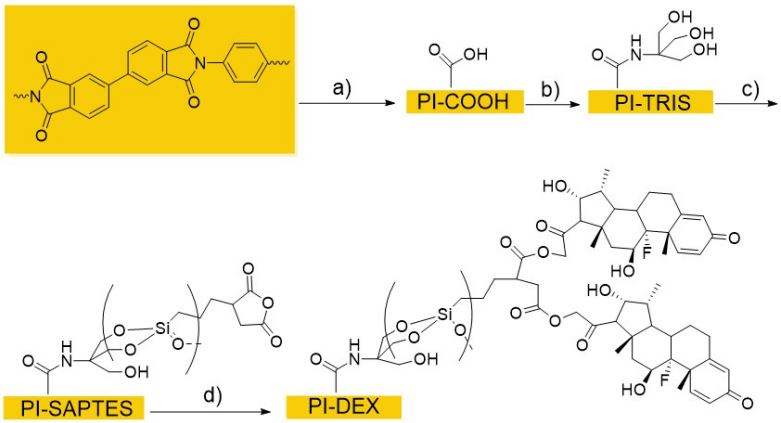

Figure 1. Synthetic pathway for the functionalization of PI films; a) KOH 5M, 50°C, 10'; then HCl 0.5M 5'; then H2O, 37°C, overnight; b) DCC, TRIZMA® base, EtOH, 50°C, 24h; c) 4% v/v solution of TESPSA, toluene, r.t., 72h; d) DCC, DMAP, ACN, 50°C, 96h.

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Cyclodextrin Suxibuzone Conjugates as proteasome activators

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Keywords: Cyclodextrin, Suxibuzone, Proteasome

The treatment of neurodegenerative diseases, such as Alzheimer's, Amyotrophic Lateral Sclerosis and Parkinson's disease, presents a significant challenge today due to their incurable and the rising incidence of these conditions [1]. Recent research has proven that these diseases are associated with protein dyshomeostasis, resulting from a compromised proteostasis network. In light of this interest, a focus has been on repurposing existing drugs to enhance proteasome activity, which could protect neurons from amyloid toxicity. This approach avoids failure in drug discovery and reduces the time and cost of marketing the drug [2].
In recent studies, the pyrazolone family of drugs, commonly used for their anti-inflammatory properties, was investigated as proteasome activators. Among these, aminophenazone, 4-aminoantipyrine and nifenazone exhibited significant efficacy in activating proteasome activity. Conversely, suxibuzone showed moderate activity [3]. Cyclodextrin was selected to increase suxibuzone effectiveness. Cyclodextrins have favorable properties, including bioavailability, low toxicity, good solubility in physiological conditions and ease of functionalization [4].
In this study, a conjugate of β-cyclodextrin and suxibuzone (Figure 1) was synthesized via a condensation reaction. Its capability as a proteasome activator was evaluated and compared to the inclusion complex of cyclodextrin/suxibuzone. The assay was carried out on the human purified 20S proteasome by measuring chymotrypsin-like activity. The results indicated that both formulations behave as proteasome activators. Notably, the inclusion complex β-cyclodextrin/ suxibuzone exhibited substantial activity starting at a concentration of 5 μM while the cyclodextrin-suxibuzone conjugate demonstrated a marked improvement, enhancing activity even at a lower concentration of 1 μM compared to suxibuzone alone. Furthermore, MTT assays confirmed the absence of toxicity in differentiated neuroblastoma cell line SH-SY5Y for all the compounds tested, suggesting that these systems could offer a promising approach to managing protein dyshomeostasis.

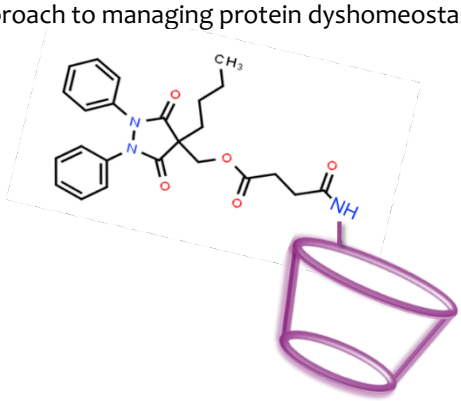

Figure 1. Cyclodextrin-Suxibuzone conjugate

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Symmetric and Asymmetric Glucose/O2 enzymatic fuel cell: effects of electrode size on power output performance

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Keywords: Enzymatic fuel cells, glucose oxidase, stencil printing

Enzymatic fuel cells (EFCs) have been proposed to catalyze oxidation of fuels at anodes and/or reduction of oxidants at cathodes and provide electrical power. EFCs can be divided into two main groups: 1) mediated electron transfer (MET) devices, in which redox species are used to transport the electrons between enzyme and electrode surface; 2) direct electron transfer (DET) based biodevices, where the enzyme is able to communicate directly with the electrode [1]. Moreover, there are some issues in defining the power output, especially considering anodes and cathodes with different sizes. For example, a $H2/O_2$ EFC combines a 6 cm^2 bilirubin oxidase-modified cathode with a 1.2 cm^2 hydrogenase-modified anode to balance the catalytic performance as a function of the H2-air mixture used [2]. The raw power output must surely now be reported in all cases? It now seems valuable to define the volumetric power of bioelectrodes in mW/cm^{-3} [3].

The aim of this work is to study the influence of electrode size on the performance of glucose/O_2 enzymatic fuel cell (EFC). The proposed EFC will encompass an efficient MET bioanode, employing glucose oxidase (GOx) modified graphite printed electrode (all materials will be enclosed in a newly formulated ink), and a DET biocathode, consisting of bilirubine oxidase (BOD) modified graphite printed electrode [4,5]. The EFCs will be assembled symmetrically and asimmetrically using three different electrode sizes: (a) 0.1 cm2, (b) 1 cm^2 and (c) 10 cm^2. All Power output will be normalized by the EFC volumes instead of the electrode areas. Finally, the EFCs were tested in human serum and saliva to explore their Limit of Detection (LOD) and their potential application as self-powered glucose biosensor integrated in wearable devices.

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Curcumin and piperine loaded-ethosomes for the prevention of pollutant-induced skin damage

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Keywords: Curcumin, piperine, ethosomes

Curcumin (CUR) is a polyphenol derived from the rhizome of the East Indian plant *Curcuma longa*, it has many pharmacological activities, including anticancer, antimicrobial, anti-inflammatory and antioxidant. In addition, it is also proven to have potential in the treatment of several skin conditions. However, its low bioavailability hampers its possible application in therapy. Piperine (PIP) is an alkaloid isolated from the seeds of *Piper nigrum*, and beside its wide range of therapeutical properties, it is known as a biopotentiator, being able to increase the bioavailability and biological effectiveness of co-administered drugs. In order to improve CUR bioavailability, it was therefore planned to deliver it within ethosomes and to study its combination with PIP.
Ethosomes (ETs) are nanovesicular drug delivery systems built up for dermal and transdermal delivery of drugs. Their composition is very simple, consisting of phosphatidylcholine, water, and ethanol in a relatively high concentration (20–45% v/v). The presence of ethanol improves the vesicle thermodynamic stability and loading capacity of lipophilic drugs in addition to increasing drug penetration through *stratum corneum*, that represents a limiting step for topical drug delivery.
The objective of the present investigation is to evaluate CUR and PIP (separately loaded into ETs) capability to prevent skin damage induced by environmental pollution.
ETs were produced and characterized for their size, morphology, and drug encapsulation efficiency. In vitro diffusion tests highlighted that ETs can promote the skin permeation of CUR. Moreover, the combination of CUR and PIP was found to increase the antioxidant power of CUR in antioxidant activity test. Finally, *ex vivo* studies were performed, using human skin explants pre-treated with the ETs formulations and then exposed to diesel engine exhaust (DEE), one of the most aggressive pollutants. Immunohistochemical analysis have shown the suitability of drug-loaded ETs to prevent skin structural damage induced by DEE exposure [1].

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Polyesters blends nanoformulations for usnic acid encapsulation

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Keywords: poly-L-lactide, polyglycerol adipate, usnic acid, drug delivery

Polymeric nanosystems offer immense potential for drug delivery by providing a versatile platform that can be finely tuned for various therapeutic applications, notably in drug delivery where they may allow for drug targeting and controlled release[1]. The present study investigates the use of nanoparticles (NPs) based on poly-L-lactide (PLLA) and polyglycerol adipate (PGA), alone and blended (50/50), for the encapsulation of usnic acid (UA), a natural compound with antimicrobial and anticancer activities, suffering from limited aqueous solubility and hepatotoxicity. Polymer NPs with sizes ranging from 100 to 150 nm were obtained by nanoprecipitation. PLLA-PGA (50/50) NPs were smaller in size and more susceptible to enzymatic degradation than pure polymers. FTIR-ATR and DSC analyses evidenced a partial miscibility of the PLLA-PGA (50/50) blend. All systems were able to encapsulate UA effectively, increasing drug apparent solubility. DSC measurements evidenced that UA was partially encapsulated in an amorphous state. That presumable contributed to the observed drug increased solubility. *In vitro* cytotoxicity tests performed on HepG2 cells showed a significant decrease in drug toxicity when encapsulated in polymers compared to free UA. The observation of cell cytoskeleton morphology evidenced that cells treated with UA-loaded nanoparticles were well arranged and formed a complex network, with several cell–cell interactions confirming the protective role of UA incorporation in nanoparticles. Overall, this research opens new avenues for the effective utilization of these highly degradable and biocompatible PLLA-PGA blends as nanocarriers for reducing UA cytotoxicity[2].

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Bioluminescence on-a-chip platform for multiplexed biosensing

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Keywords: Bioluminescence, 3D models, tissue-on-a-chip, biosensing

Living cells used as sensing systems have proved to be valuable tools for prediction of the physiological response to drugs, chemicals, and samples in complex matrices, which toxic effects and specific biological activities can be evaluated in an easy and straightforward manner. Thanks to their superior predictivity, 3D cell models (i.e. spheroids, organoids and microtissues) are increasingly replacing conventional 2D cell cultures, enabling to recapitulate the extracellular matrix and cell-cell interactions and creating an architecture that faithfully reflects the native morphology of organs and tumors. Bioluminescent reporter assays represent the gold standard for several high throughput screening assays employed in drug discovery and BL proteins showed a formidable tool for elucidating the biological mechanisms underlying morphogenetic and pathogenetic processes and for unravelling molecular pathways involved in the etiopathogenesis of several diseases. In accordance with the three key pillars of the organ-on-a-chip technology, we developed a bioluminescent tissue on-a-chip, relying on different cell lines (i.e., human embryonic kidney (HEK293T), human cervical cancer (HeLA) and human dermal fibroblast (HDF) cells) genetically engineered with newly developed luciferase mutants emitting at different wavelengths and characterized by high stability, implemented in a microfluidic system for multiplexed biosensing [1,2]. Thanks to 3D printing technology a cell cartridge and an adaptor was developed to provide a mini-dark box interfaced with portable light detectors (i.e., smartphone and CCD camera) for BL signals acquisition.

To the best of our knowledge this is the first implementation of a bioluminescence in a tissue-on-a-chip. The proposed biosensing platform could become a useful tool for multiple bioactivity analysis, for an initial on-site screening of potentially toxic substances, prioritizing samples for a more accurate chemical analysis.

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A new chiral peroxidase-like activity guanosine self-assembled hydrogel biosensor based on circularly polarized chemiluminescence emission for H2O2 detection and quantum computing

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Keywords: Circularly polarized chemiluminescence, H2O2, guanosine self-assembled hydrogel, Smartphone-based biosensor, Quantum computing

Chemiluminescence (CL) is widely used for hydrogen peroxide detection, mainly exploiting the highly sensitive peroxidase-luminol-H2O2 system. Hydrogen peroxide plays an important role in several physiological and pathological processes and is produced by oxidases, thus providing a straightforward way to quantify these enzymes and their substrates [1]. Recently, biomolecular self-assembled materials, obtained by guanosine and its derivatives and displaying peroxidase enzyme-like catalytic activity, have received great interest for hydrogen peroxide biosensing [2]. These soft materials are highly biocompatible and can incorporate foreign substances while preserving a benign environment for biosensing events. In this work, a guanosine hydrogel prepared using a mixture of guanosine and guanosine 5′-monophosphate in the presence of K^+ ions is loaded with luminol and hemin to produce a functional material showing peroxidase-like activity to the CL reaction of luminol with H2O2. The hydrogel is then functionalized with a specific oxidase enzyme to enable marker biosensing: the hydrogen peroxide produced by analyte oxidation reacts, in the presence of the self-assembled guanosine/hemin gel mixture, with luminol to produce photon emission. The biosensor takes advantage of the features both of CL detection, offering high detectability and amenability to miniaturization, and of the 3D porous structure of hydrogel, as providing increased stability and catalytic activity even in alkaline and oxidizing conditions of incorporated enzymes. We developed four different guanosine hydrogel – based biosensors for the detection of glucose, xanthine, uric acid and lactate by simple incorporation of the corresponding oxidases: glucose oxidase (GOx) [3], xanthine oxidase (XOD), urate oxidase (UO) and lactate oxidase (LOx). Exploiting 3D printing technology, smartphone-based portable devices for CL biosensors were developed, verifying their applicability to quantify biomarkers of clinical interest at the point-of-care (POC). The photons emission was detected using a portable device that employs a smartphone's CMOS camera for CL emission detection. Once the chirality of the hydrogel was verified through circular dichroism, we demonstrated for the first time both experimentally using circularly polarized fluorescence (CPF) and by *ab initio* quantum mechanical calculations that the structural chirality of the gel circularly polarizes the CL emission and the electronically excited singlet state of 3-aminophtalate. The polarization degree of freedom of photons can be used to encode information, and this information is typically manipulated using so-called half-wave plates and polarizing beamsplitters. In our case, CL photons are manipulated by the chiral environment. With respect to its polarization, a CL photon can be considered as a qubit, paving the way to the realization of a biomimetic biosensor for quantum computing applications [4].

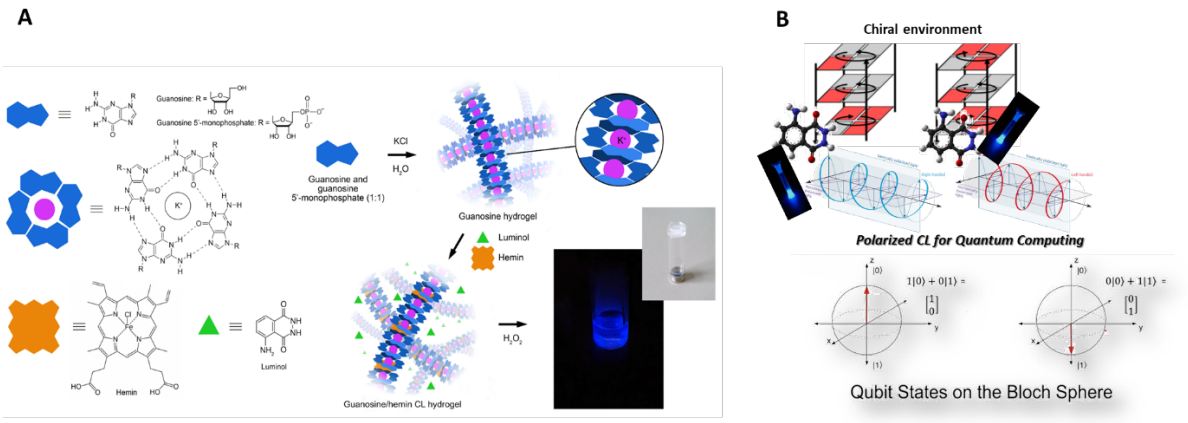

Figure. (A) Scheme of the formation of the guanosine/hemin CL hydrogel, by supramolecular self-assembly of guanosine and guanosine 5′-monophosphate in the presence of K^+, hemin, and luminol. (B) Equivalence of two quantum states of an Optical Qubit as right-handed and left-handed circularly polarized CL emissions of luminol modulated by chirality of supramolecular self-assembly of guanosine hydrogel.

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Acknowledge

Progetto di ricerca PRIN 2022 "Biomimetic sensing platforms for the detection of Alzheimer's disease related biomarkers", Codice Progetto 2022WN89PC, CUP J53D23007670006, finanziato nell'ambito del Piano Nazionale di Ripresa e Resilienza PNRR - Missione 4 – Componente 2 – Investimento 1.1 "Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)" (Bando indetto con D.D. del MUR n. 104 del 02/02/2022).

AMYC BIOMED 2024

Discovery and development of diterpene-based inhibitors of ArnT-mediated colistin resistance

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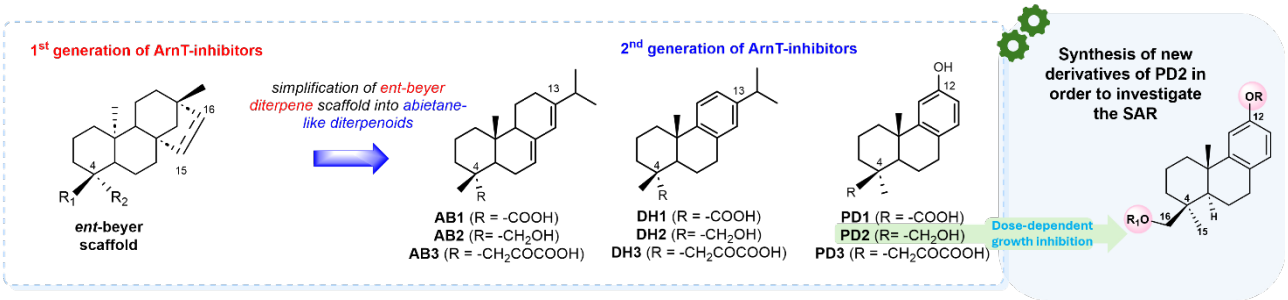
Keywords: colistin, antimicrobial resistance, ArnT inhibitors, abietane diterpenoids

Colistin, also known as polymyxin E, is a last-line antibiotic used for the treatment of multidrug resistant Gram-negative bacterial infections.[1] However, in recent years, the occurrence of resistance phenomena related to the use of such antibiotics has been documented. The molecular mechanism that gives resistance to colistin in several Gram-negative bacteria, including *Pseudomonas aeruginosa*, has to be mainly ascribed to a glycosyltransferase enzyme, named ArnT.[2] In a previous study, by combining microbiological assays and molecular modeling, we have been able to demonstrate that a diterpene scaffold is a promising platform for the development of novel ArnT inhibitors.[3] In order to further optimize this scaffold, we set up a rational procedure that simplifies the *ent*-beyerane scaffold into drug-like synthetic molecules able to minimize the pharmacophores that proved to be crucial for biological activity. As a result, different naturally occurring diterpene scaffolds have been evaluated as a source of starting material for the synthesis of a second generation of ArnT inhibitors. In particular, we have selected the abietic, dehydroabietic and podocarpic acid (AB1, DH1, PD1) as some key starting points. Furthermore, with the aim of enlarging the abietane derivatives library, the corresponding hydroxylated (AB2, DH2, PD2) and oxalated (AB3, DH3, PD3) derivatives have been synthesized, tested against colistin-resistant *Pseudomonas aeruginosa* strain and their binding mode was investigated through molecular docking simulations highlighting PD2 as the most promising compound able to restore colistin sensitivity in bacteria, showing a dose-dependent growth-inhibition. Thus, our efforts are focused on the synthesis of new derivatives of PD2 with the aim to investigate the SAR of the podocarpic scaffold. In particular, different derivatives of PD2 have been synthesized with the aim of investigating the role of (i) the oxalyl group at C-16, an essential requirement for the ArnT activity inhibition of the hit compound; (ii) the length and flexibility of the alkyl chain of the functional group at C-16; (iii) the presence of a sugar unit to mimic L-Ara4N; (iv) the substitution of the oxygen with nitrogen heteroatom in the functional group at C-16 on the biological properties of the original abietane scaffold; and (v) the role of the phenolic group at C-12.



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AMYC BIOMED 2024

Melanins as bio-inspired photocatalysts for reductive dehalogenations of α-halogenated carbonyl compounds

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Keywords: photocatalysis, melanins, dehalogenation, radical reactions

Melanins are a wide group of natural pigments differing for composition, occurrence and functions, having common origin from oxidative polymerization of phenolic derivatives. Melanins can be classified in five main groups: eumelanin, pheomelanin and neuromelanin found in mammals, allomelanin found in plants and fungi, and pyomelanin found in bacteria. [1] Given the well-documented antioxidant properties exhibited by melanins via electron transfer and/or H-atom transfer mechanisms, [2] we decided to focus our attention on a set of model photocatalyzed reductive dehalogenations of α-halogenated carbonyl compounds (see Fig.1) [3]. In our study we tested four different synthetic melanin polymers: DHI-melanin (**MEL^{DHI}**) and DHICA-melanin (**MEL^{DHICA}**), obtained from 5,6-dihydroxyindole (DHI) and its 2-carboxylic acid (DHICA), respectively, and inspired to mammalian eumelanins; DHN-melanin (**MEL^{DHN}**), obtained from 1,8-dihydroxynaphthalene (DHN) and inspired to fungal allomelanins; and polydopamine (**MEL^{DA}**), obtained from dopamine and inspired to the high adhesive *Mytilus edulis* foot proteins (Mefps) produced by mussels [4].
The reactions were performed under stirring at room temperature in MeOH under inert atmosphere (N_2 bubbling), upon irradiation with a LED lamp (Kessil, 427 nm, 40W power) for 24 hours using sodium ascorbate as reducing agent. Reaction monitoring was based on GC-MS analysis of the crude reaction mixtures, while compounds identification and quantification was realized by comparison with authentic samples.
We demonstrated that melanin polymers are competent materials in the photocatalyzed reduction of α-halogenated carbonyls when irradiated with a violet LED lamp in the presence of sodium ascorbate as the reductant. The best performing melanin was the **MEL^{DHICA}**, which allowed to dehalogenate efficiently diethyl bromomalonate (**1-Br**), dimethyl bromomalonate (**2-Br**), and phenacyl bromide (**3-Br**) with the expected products formed in 90, 85 and 66% yield, respectively; on the other hand, diethyl and dimethyl chloromalonate esters (**1**-Cl and **2-Cl**) were almost unreactive under the same reaction conditions; we attribute this behavior to their strongly negative reduction potential. Electrochemical and control experiments enabled to elucidate the reaction mechanism, demonstrating the key role of electrons as charge carriers in the observed photocatalytic process.
In conclusion, the present work highlights that melanin polymers can be considered as bio-inspired photocatalysts in synthetic endeavors, representing an additional tool toward the development of sustainable methodologies. The successful application of synthetic melanins in these reactions could contribute to waste treatment and disposal of halogenated organic materials by means of sustainable photocatalysts [3].

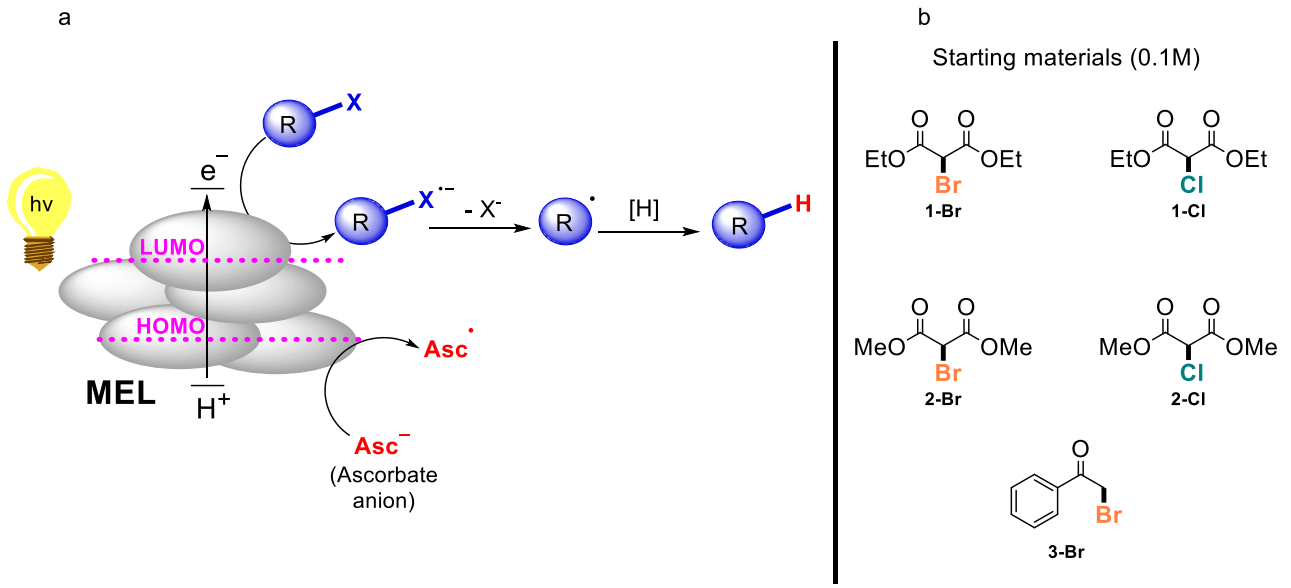

Fig. 1. a) Mechanistic proposal of α-halo carbonyl dehalogenation reactions using melanins in the role of heterogeneous photocatalyst. b) Starting materials: diethyl bromomalonate (**1-Br**), diethyl chloromalonate (**1-Cl**), dimethyl bromomalonate (**2-Br**), dimethyl chloromalonate (**2-Cl**) and phenacyl bromide (**3-Br**), respectively.

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We thank the MUR for financial support (PRIN 2020 Project "Combined Electrochemical and Light-Driven Processes for the Sustainable Synthesis of Added-Value Molecules (ElectroLight4Value)"; Code: 2020927WY3).

Pyro-electrohydrodynamic printing of biomolecule microarrays

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Keywords: biomolecule microarray, diluted samples, pyro-electrohydrodynamic jet

To date, the quantification of low abundant biomolecules has achieved a relevant role in biological assays. In particular, the printing of biomolecule microarrays is revolutionizing the clinical procedures based on the classical biochemical and immune assays thanks its possibility to analyze more of thousands diagnostic entities within a single experiment and using a very small sample volume. Moreover, the printing procedure can also avoid invasive procedures for collecting biological samples due to its great sensitivity in the search for biomolecules even from peripheral fluids enhancing the patients compliance. Herein an innovative system based on the phenomenon of pyro-electrohydrodynamic jetting (p-jet) is presented with the aim to demonstrate its capability to accumulate, with high efficiency, a high number of tiny biological droplets (down to 1 pL) [1] on a chemical activated glass surface. Taking advantage of the pyroelectric effect biological samples and to read solutions down to low pg/mL [2] which are difficult to detect, with the classical microcontact printing techniques [3]. In particular, the biomolecule generated spots exhibit high reproducibility with a coefficient of variation (CV) below to 10%, both in diameter and fluorescence intensity. In the end, we think that through the compact and cost-effective of our system, it could be a real promise in the field of sensing and in particular for the detection of highly dilute biological samples.

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Strategic amino acid substitutions in the primary structure of the antimicrobial peptide Esc(1-21) to modulate its biological properties

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Keywords: antimicrobial peptides, amino acid substitution, antimicrobial resistance, pseudomonas aeruginosa, staphylococcus aureus

The search for new antimicrobial compounds is extremely pressing, considering the increase in number of microbial infections resistant to conventional antibiotics. Antimicrobial peptides (AMPs) of innate immunity represent interesting alternative to antibiotics with expanding properties. Esc(1-21) [GIFSKLAGKKIKNLLISGLKG-NH2] is a short-size AMP from frog skin with a potent activity against *Pseudomonas aeruginosa* but with a weak potency against the Gram-positive *Staphylococcus aureus* [1]. We therefore designed two analogs of Esc(1-21), by replacing strategic amino acid residues in order to improve the antimicrobial/antibiofilm activity of the parent peptide. In the first case, Leu^{14} and Ser^{17} were replaced with the corresponding D-enantiomers and the obtained peptide, named Esc(1-21)-1c resulted to have (i) an higher biostability; (ii) higher antibiofilm activity and (iii) lower cytotoxicity; (iv) higher in vivo efficacy [2]. In a second case, Gly^{8} was replaced with the non-conventional residue α-aminoisobutyric acid and the designed peptide, named [Aib^{8}]-Esc(1-21) resulted to be (i) more active against the planktonic form of Gram-positive bacterial strains, especially *Staphylococcus aureus*, including multidrug-resistant clinical isolates, (ii) more stable with the respect of the parent peptide without resulting cytotoxic to mammalian cells; (iii) more active against the biofilm form of *S. aureus*. Overall, these results have shown how strategic amino acid substitutions are sufficient to enlarge the spectrum of activity of the parent peptide Esc(1-21), and improve its biological properties for therapeutic purposes.

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Acknowlegments:
This research was supported by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) to M.L.M and Fondazione Italiana per la Ricerca Cistica (Project FFC#4/2022) Delegazione FFC Ricerca di Roma e della Franciacorta e Val Camonica

Improving the sensitivity and the cost-effectiveness of a competitive visual lateral flow immunoassay through serial designs of experiments

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Keywords: Cortisol, gold nanoparticles, experimental design, rapid test, point-of-care test

The colorimetric Lateral Flow Immunoassay (LFIA) based on the visual read-out interpretation is the most diffused point-of-care test. Despite its strong field establishment in the biosensing panorama, a drawback is the low sensitivity. Nevertheless, a systematic unbiased optimisation of the experimental conditions is rarely performed to maximise the sensitivity of the available material for an LFIA device development. The need of many experiments and bioreagents (antibodies and antigens) consumption discourages the exhaustive exploration of the influent variables. On the other hand, by means of an appropriate strategy, the number of required experiments can be restricted. In fact, for some non-competitive LFIA this approach led to a significant increase in the sensitivity [1]. In this work we applied an experimental-design-based optimisation on a competitive LFIA. We aimed at improving the sensitivity of a competitive LFIA for the detection of cortisol. The sensitive detection of cortisol is clinically relevant (2.0-10ng/mL in saliva, higher with Cushing syndrome or stress related diseases [2,3]). The LFIA includes the antigen (cortisol-BSA) spotted onto the test line, a labelled monoclonal antibody as the signal reporter and ruby red gold nanoparticles as the label (Ab_AuNP) (**Figure 1**). The idea underlying optimization process was the execution and the elaboration of one or more progressive designs of experiments (DoE), up to achieving the maximal sensitivity with the available materials. Taking inspiration from the ELISA theory [4], we considered, as the variables, the amount of Ab conjugated with gold nanoparticles (antibody, Ab), the amount of gold conjugate in the assay (optical density, OD), the concentration of the antigen on the test line (T). After each experiments the test line colour intensity was acquired by a scanner. A key role for making proper decisions along our progression, had the definition of a convenient figure-of-merit for the evaluation of each DoE. Therefore, each combination was tested in the absence (NEG, true if > 80 a.u., false otherwise) and in the presence of cortisol (POS, true if < 15 a.u., false otherwise). At the end of the optimization process standard curves were performed and the starting and optimized LFIAs were compared in terms of analytical features and reagent consumption. The differently optimized conditions were named LFIA 0 (unoptimized), LFIA 1 (optimized after 1 DoE, 13 experiments), LFIA 2 (optimized after 3 DoEs, 34 experiments). Compared to LFIA 0 (vLOD >10µg/mL; reagent consumption 400ng of antibody and 480ng of antigen), the sensitivity increased more than 500-fold in the LFA 1 (vLOD=20.5 ± 1.0 ng/mL), and 5000-fold in the LFIA 2 (vLOD=2.2 ± 0.1 ng/mL), while the reagents consumption decreased by a factor of ca9 (20.6ng antibody and 80ng antigen) and ca37 (3.6ng antibody and 20ng antigen) in the LFIA 1 and LFIA 2, respectively. This demonstrates the power of a systematic unbiased approach.

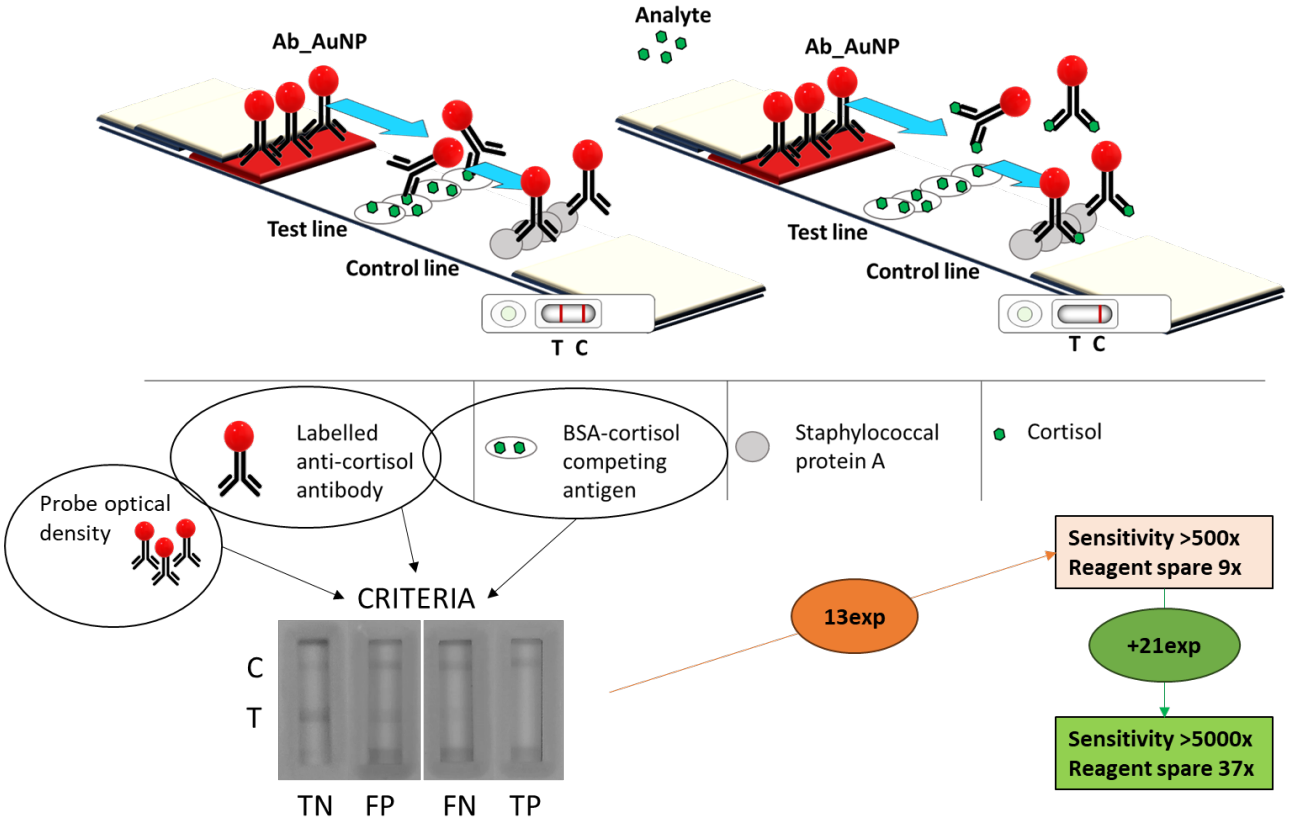

Figure 1: The scheme of the competitive lateral flow immunoassay. The three variables (optical density of the probe, modification index of the antibody, and test line antigen concentration were optimized through serial experimental designs evaluated on the basis of test line color intensity resulting in true/false positive/negative responses as the decision criteria. On the bottom right the improvement sensitivity and reagent spare) after partial and full optimization process.

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AMYC BIOMED 2024

Spirulina-derived phytochemicals for applications in optical and fluorescent sensing

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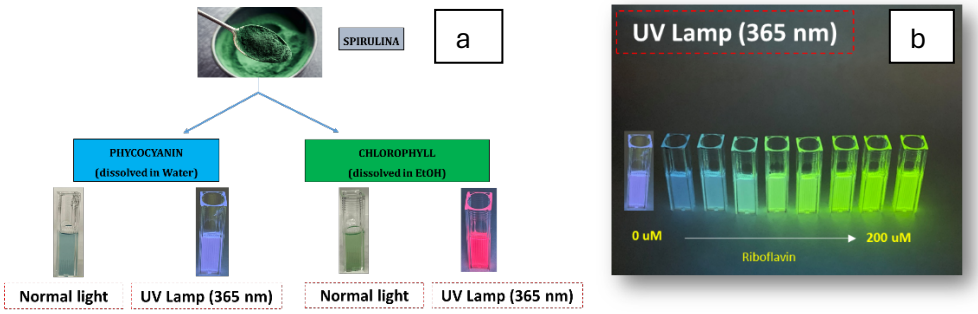
Keywords: Spirulina, Phytochemicals, Chlorophyll, Phycocyanin, Optical and fluorescent sensors

Phytochemicals are biologically active organic substances present in plants. Among the wide variety of plants known, Spirulina Platensis, is a microalgae which has been largely investigated for its biological and health beneficial properties, being rich in various phytochemical compounds including polyphenols, alkaloids, terpenoids, phycocyanin, chlorophyll, flavonoids, tannins, quinones [1].

In this report, we extend the study to the optical and fluorescent properties of two main classes of phytocompounds presents in the Spirulina plant, i.e. chlorophyll and phycocyanin with the main objective to develop optical and fluorescent sensors. This aspect has not been adequately investigated until now and only very few papers are reported in the literature [2]. Chlorophyll and phycocyanin were extracted by a simple procedure from Spirulina powder and their optical and fluorescent properties were analyzed (see Scheme 1a).



Scheme 1. a) Optical and fluorescent properties of the phycocyanin and chlorophyll components extracted by Spirulina powders. b) Fluorescent properties of phycocyanin in presence of riboflavin.

Both components display high fluorescence intensity which can be applied for developing heavy metals and biomolecules fluorescent sensors. In Scheme 1b is shown an example related to the monitoring of riboflavin (Vitamin B2) exploiting the naked eye change of phycocyanin fluorescence.
The development of solid-state sensors on paper or plastics based on these methods and the aid of a smartphone is currently in progress.

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Synthesis and in vitro study of new derivatives for transthyretin cardiac amyloidosis treatment

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Keywords: amyloidosis, small molecules, transthyretin, stabilizers, ATTRs

Transthyretin (TTR) is a β-sheets-rich homotetrameric protein characterized by four monomers arranged together around a two-fold axis. The acronym TTR holds the main protein's physiological functions: transporter, thyroxine (T_4) and retinol, the latter through the binding with the retinol-binding protein (RBP). TTR tetramer is crossed by a channel divided into two different cavities, named thyroxine-binding pockets (T_4BP). In contrast with its physiological role, TTR possesses an intrinsic amyloidogenic potential related to its high level of the β strand. Under pathological conditions, TTR undergoes a misfolding process that leads to the formation of protein aggregates and fibrils, in the tissues, provoking the organ damage and dysfunction and inducing amyloidosis disease onset [1]. TTR is responsible of several amyloidosis diseases (ATTRs), such as senile systemic amyloidosis (SSA), related to wild-type TTR (wt-TTR); amyloid cardiomyopathy (AC) and amyloid polyneuropathy (FAP) caused by TTR mutants, especially V30M and V122I [2].

One of the therapeutic approaches against ATTRs progression is the use of small molecules able to bind the T_4BP, contributing to the stability of the TTR tetramer. Tafamidis is the first-in-class drug approved for the treatment of TTR amyloid cardiomyopathy, and it is still the only drug used in a clinical setting [3].

In this context, we propose the synthesis and characterization of a new series of di-aryl derivatives (**1a-n**,**2a-n**,**3a-n**) as potential TTR stabilizers. The results of the turbidimetric and thioflavin T (ThT) assays suggested that compounds **1a**, **1d**, **2n** and **3g** showed a promising profile both on wt-TTR and mutants (V30M, V122I). Thus, the binding mode of these four derivatives were investigated combining different techniques: ANS fluorescence displacement binding assay, isothermal titration calorimetry (ITC) and the X-ray analysis, Figure 1.

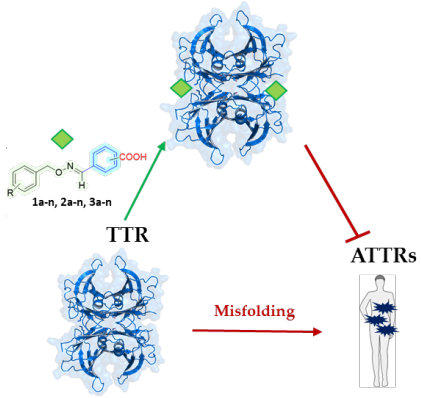

Figure1 Graphic summary

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Novel snail-mucus-extract-coated gold-nanoparticles for potential skin cancer prevention and treatment

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Keywords: snail mucus, inflammation, antioxidants, UV-filters, melanoma

Recent studies have demonstrated the ability of snail mucus to maintain healthy skin due to its emollient, regenerative, and protective properties. Specifically, mucus from *H. aspersa muller* has shown benefits such as antioxidant and antimicrobial activities, as well as wound healing capabilities [1,2]. To enhance the antioxidant activity of snail mucus, it was extracted using a hydroalcoholic solution and subsequently freeze-dried. This resulted in a snail mucus extract (SME) with increased antioxidant activity in cell-free assays. However, testing its effects on cellular models proved challenging due to the formation of a thick film on cell surfaces. To extend the benefits of snail mucus and broaden its applications, SME was used to develop snail mucus extract-coated gold nanoparticles (AuNPs-SME), which exhibited anti-inflammatory properties in non-tumorigenic cells. The cytoprotective effects of these nanoparticles were evaluated using an LPS-induced inflammation model in human NCTC keratinocytes. Co-treatment with LPS and AuNPs-SME significantly reduced the transcription of pro-inflammatory cytokines. Additionally, we demonstrated that AuNPs-SME can serve as an anti-inflammatory treatment, as well as a sunscreen and antioxidant for potential cosmeceutical applications. Furthermore, AuNPs-SME selectively inhibited the growth of two human melanoma cell lines without affecting the viability of immortalized human keratinocytes under the same conditions. Innovative nanotechnologies, such as the use of gold nanoparticles, offer promising advancements in cancer treatment by providing targeted drug delivery, reducing side effects, and enhancing the efficacy of therapeutic agents [3-5]. Thus, we have shown that snail mucus is a suitable candidate for innovative formulations, enriching the snail mucus-based anti-aging and sunscreen products already available on the market. Moreover, novel formulations containing snail mucus have potential applications in treating specific cancers, particularly melanoma, through advanced nanotechnological approaches.

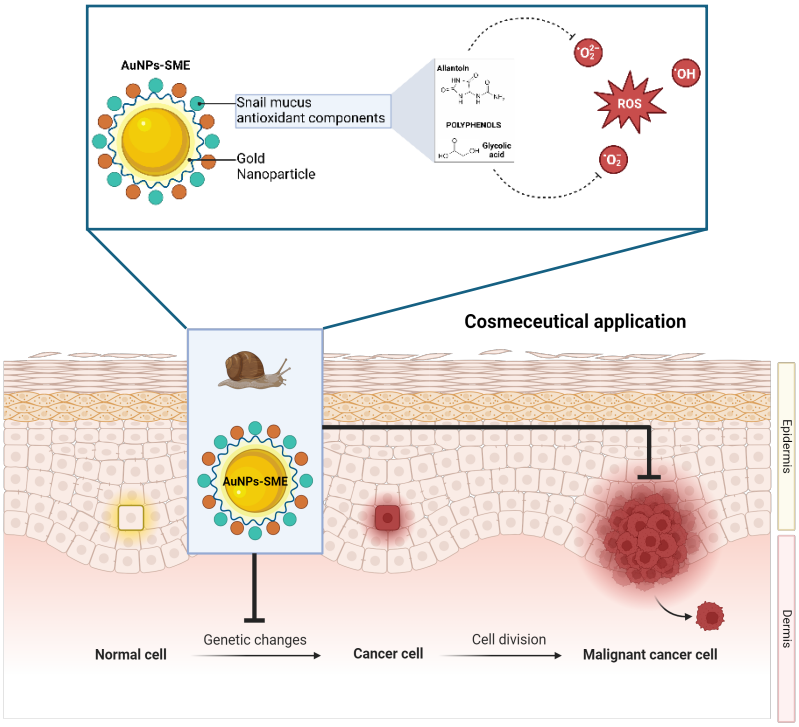

Figure 1. Illustration of AuNPs-SME mechanism of action on keratinocytes and melanoma cells.

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Neuroprotection Activity of Different Novel Epigenetic Modulators: A New Therapeutic Strategy in Retinitis Pigmentosa

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Keywords: neurodegeneration; retina; epigenetic modifiers, nuclear enzymes

Epigenetic modifiers are increasingly being investigated as potential therapeutics in neurodegeneration. Because neurons in the nervous system are postmitotic, diseases of the nervous system offer a distinct substrate for epigenetic alteration. In this context, the retina is a valuable model for studying the epigenome's role in both normal and pathophysiological conditions.
The expression of genes in photoreceptors is controlled by transcription factors and alterations in epigenetic regulation, including modifications to histones [1].
The enzymes lysine demethylase 1 (LSD1), histone deacetylase 6 (HDAC6), and histone deacetylase 1 (HDAC1), responsible for removing specific histone modifications, play significant roles in the development of rod photoreceptors. In animal models of Retinitis Pigmentosa, the inhibition of these enzymes led to a notable reduction in rod degeneration, with consequent preservation of vision, and increased expression of neuroprotective genes. This included downregulating genes associated with inflammation and cell death [2].
In this study, different types of epigenetic modulators were tested *in vitro*, where their inhibition of the enzyme and their effectiveness in preserving cell viability after oxidative stress damage were evaluated. Next, the HDAC6 enzyme modulator (namely Repistat), which was found to be the most effective *in vitro*, was tested on an animal model of RP, the *rd10* mouse. Repistat efficacy was evaluated by electrophysiology (Electroretinogram), immunohistochemical, and biochemical (Western Blot and Real-Time PCR) techniques. Repistat was found to be effective in preserving the function and morphology of photoreceptors and thereby delaying their degeneration.

Founded by PRIN 2022 "Novel epigenetic modifiers for a mutation-independent pharmacological approach to extend photoreceptor survival in Retinitis Pigmentosa – RePiSTOP" – CUP: I53D23004230001

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DISCOVERY OF NOVEL PIPERIDINE DERIVATIVES AS HIGH SELECTIVE SIGMA 1 RECEPTOR LIGANDS

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Keywords: sigma receptors, antagonists, neuropathic pain, piperazine, piperidine.

Neuropathic pain (NP) arises as a direct result of damage or disease affecting either the peripheral or central somatosensory nervous system. The effectiveness of the treatments used to manage NP is often variable and conventional drugs are mostly characterized by uncertain efficacy [1]. The sigma-1 receptor (S1R) represents a promising target for the treatment of NP with S1R antagonists having analgesic action in *in vivo* NP animal models[2]. Prompted by continuous interest in the development of molecules for the treatment of pain, the aim of present study was to develop new potent and selective ligands for S1R able to produce *in vivo* analgesia. Thanks to structure-affinity relationship studies, ten *N*-methylpiperidine and piperazine derivatives – characterized by the presence of a basic amine flanked by two hydrophobic groups – have been developed (**Figure 1**). Binding affinities were evaluated over S1R and sigma-2 receptor (S2R) through radioligand assays [3].

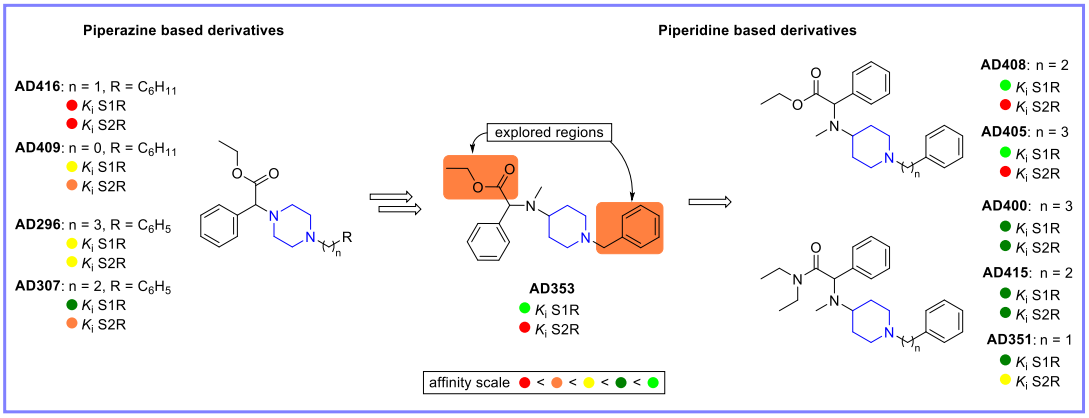

Figure 1. Structure and binding affinities of piperidine and piperazine derivatives.

Variations in binding affinity were assessed upon alteration at three specific positions: substitution of the aromatic ring (1); modification of the length of the side chain linked to the heterocyclic nitrogen (2); introduction of a free tertiary amine (3). This SAR studies led to the selective S1R compound AC1 which has shown neglected *in vitro* cytotoxicity and low K^+hERG inhibition (IC50 = 1.6 μM). Molecular modelling studies and evaluation on *in vivo* NP mouse models are currently in progress.

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Bioconjugates of ferritin with Au(I) as anticancer agents

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Keywords: Human ferritin; nanocarrier, metal-based drugs, anticancer compounds

Human heavy-chain ferritin (HuHf) is a recombinant protein composed of 24 H-subunits, forming a nanocage with a hollow globular structure. The H-chain is recognized by the transferrin receptor-1 (TfR1), overexpressed in many cancer cell lines. HuHf is a suitable nanocarrier due to its versatility, excellent safety profile and nano-range size[1]. Gold compounds are potential anticancers with antiproliferative and proapoptotic properties[2]. Here, I worked on the development of human heavy chain ferritin conjugates with different gold(I) compounds, i.e. Auranofin[3], Aurothiomalate and a monocarbene for the production of ferritin-based nanocarriers and their selective delivery toward cancer cells. Five human-H chain ferritins were expressed in *E. coli* cells: the wild type, three different mutants, where I replaced with Alanine one or two cysteines (C130A, C90AC102A and C90A) and a ^{19}F labelled-human ferritin (5-F-Trp93). I exploited the use of recombinant wild type HuHf for the targeted delivery of the gold(I) compounds, the three mutants to determine their binding sites on the protein and the ^{19}F-labelled ferritin as a probe for cellular uptake via ^{19}F NMR studies. For all the HuHf-gold(I) adducts: ESI-MS spectrometry (measuring the disassembled subunits) confirmed the adducts formation and provided information on the chemical nature of the species present in solution. ICP determined the total amount of gold per cage. Also, biological experiments were performed to evaluate the cytotoxicity of the free drugs and their bioconjugates with ferritin against A2780 ovarian cancer cells; moreover, the cytotoxicity of the free compounds was evaluated together with the TfR1 expression and the protein internalization in five different cell lines i.e. U87MG glioblastoma cells, MCF-7 breast cancer cells, HCT-116 colorectal cancer cells, MRC-5 fibroblast cells and HEK 293T cells.

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Synthesis and biological evaluation of novel σ1R ligands

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Keywords: drug discovery, sigma receptor, sigma receptor antagonists, chronic pain.

Chronic pain is still considered an unmet clinical need due to the limits of conventional therapies in pain relief. Alternative targets have been recently investigated, including the sigma-1 receptor (σ1R). Studies conducted on animal models of neuropathic pain showed that σ1R antagonists can reduce pain hypersensitivity. In medicinal chemistry research, normetazocine derivatives have been considered a versatile scaffold for developing new drugs. It has been established that the (−)-(2*R*,6*R*,11*R*)-configuration of the normetazocine nucleus is favored for opioid receptors interaction. Conversely, its (+)-(2*S*,6*S*,11*S*)-enantiomer exhibits different activity and showed affinity for σ1R. So, we designed and synthesized a series of novel (+)-(2*S*,6*S*,11*S*)-normetazocine derivatives to investigate the pivotal role of normetazocine stereochemistry in their pharmacological fingerprint. The most promising ligand was the σ1R selective compound (+)-LP2 (K_i = 26.61 ± 2.35 nM) tested as a diastereomeric mixture of (+)-(2*R*)-LP2 and (+)-(2*S*)-LP2. It decreased the second phase of the formalin test, showing an antagonist σ1R profile.[1] Moreover, (+)-LP2 significantly attenuates neuropathic pain by reducing central gliosis and pro-inflammatory cytokines expression levels in the ipsilateral spinal cord tissues of animals that have undergone sciatic nerve chronic constriction injury.[2]

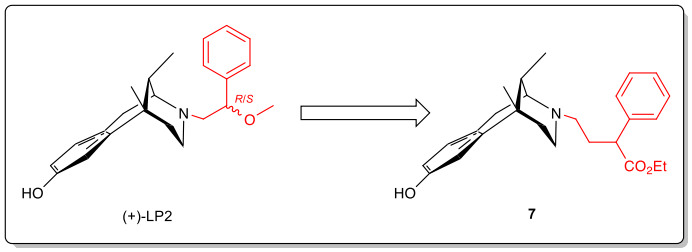

Figure 1. Chemical structures of (+)-LP2 and the newly synthesized compound **7**.

Because of its interesting pharmacological profile, a series of (+)-LP2 analogs was additionally synthesized. *In vitro*, the affinity profile of new ligands *versus* σ1R was evaluated, and compound **7** showed a relevant σ1R affinity (K_i = 27.67 ± 8.49 nM). Compound **7**, *in vivo*, also significantly reduced the second phase of the formalin test. Molecular modeling studies were also performed to analyze the binding mode and the key interactions between the new ligands and σ1R.

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AMYC BIOMED 2024

Peptidomimetics of SOCS1 and SOCS3 proteins: design and characterization

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Keywords: CLIPS, JAK/STAT pathway, SOCS1, SOCS3, cytokines

The Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway is selectively upregulated in response to cytokine stimulation in a variety of inflammatory, immune and carcinogenic processes[1]. A family of endogenous proteins negatively regulates the pathway: the Suppressors Of Cytokine Signalling (SOCS) proteins[2]; in particular, SOCS1 and 3 are downregulated in inflammatory and cancer diseases [3]. Specifically, we aimed to novel mimetics of these two proteins using previously developed peptidomimetics [4; 5] namely internal cyclic PS5(Nal1) (with a lactame bridge) for SOCS1 and the linear KIRCONG chim for SOCS3. In novel compounds we introduced non native xylene scaffolds through Chemical Linkage of Peptides onto Scaffolds (CLIPS) technique [6]. Concerning SOCS1, the thio-monocycle PS5(Nal1) and the thio-bicycle PS5(Nal1) were characterized using Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) studies (**Figure 1**) [7]. Their affinity for JAK2 was evaluated using Micro-Scale Thermophoresis (MST) and their inhibitory abilities through a phosphorylation inhibition experiment: in both assays thio-monocycle demonstrated more efficient to mime SOCS1 protein functions [7]. Contrary, KIRCONG chim was modified to enhance its water solubility by introducing polyethylene glycol (PEG) [8] and to introduce partially flexible cycles by cyclizing either only the KIR (monoleft derivatives) or the CONG (monoright analogues) portion. Similarly these mimetics were characterized by MST and preliminary results indicate that the cyclization of KIR portion led to ineffective mimetics while those bearing structural constraints in the CONG region were more efficient with a K_D value of (5±2)*10 µM.

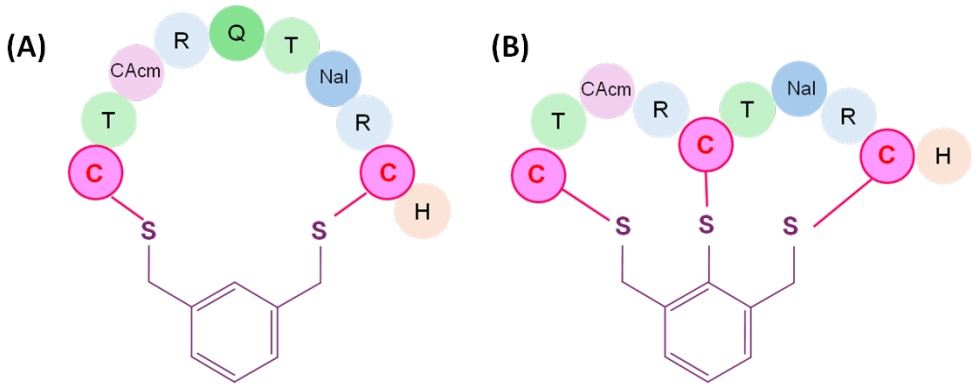

Figure 1. Schematic structure of **(A)** thio-monocycle PS5(Nal1) and **(B)** thio-bicycle PS5(Nal1).

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AMYC BIOMED 2024

Fighting glioblastoma through imidazo[1,2-*a*]pyridine-based selective ALDH1A3 inhibitors

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Keywords: ALDH1A3, anticancer, glioblastoma, optimization, synthesis.

Glioblastoma multiforme (GBM) is the most common primary malignant brain cancer, known to be a great challenge in clinics due to the extremely poor prognosis and high molecular heterogeneity it is characterized by. Even if combined with radiotherapy and surgery, chemotherapeutics are often limited by drug-resistance phenomena which significantly reduce the therapy efficacy till failure, in most cases. Also, the persistence of cancer stem cells (CSCs) confers GBM with stemness and self-renewal properties, aggressivity, in terms of invasiveness and plasticity, and therapy-escaping capabilities.[1]

Thus, CSC-targeted therapy has recently garnered attention among the new exploitable anticancer strategies and, in this context, intracellular CSC biomarkers can offer the opportunity to selectively and efficiently counteract the disease. Among them, Aldehyde Dehydrogenase 1A3 (ALDH1A3, EC: 1.2.1.3), an enzyme involved in converting aldehydes into carboxylic acids, such as retinal in retinoic acid, emerged for its key multifaced function in cancer and high druggability.[2]

In the last decade, our research group developed a library of imidazo[1,2-*a*]pyridines as potent and selective ALDH1A3 inhibitors, with some representatives, such as **NR6**, emerging for selective micromolar enzyme inhibition and antiproliferative activity on 1A3-overexpressing U87MG cells.[3,4] Driven by previous crystallographic and computational data, the effect of different substitutions on the phenyl ring at position 2 of the heterocyclic core was explored. Thus, a new series of derivatives was designed and synthesized aimed at improving both inhibitory potency and solubility. Also, their biological evaluation allowed us to construct robust structure-activity relationships and address the synthetic efforts.

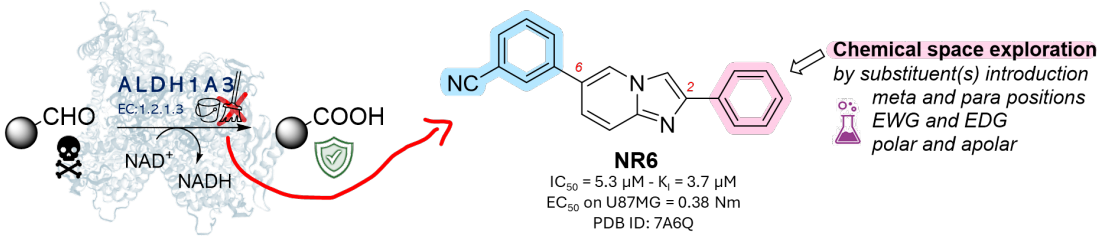

Figure 1: Detoxifying role of ALDH1A3, chemical structure of **NR6** with purple-highlighted portion investigated in this work

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DNA G-quadruplex as target for anticancer therapeutics: stability and energetics of interaction with protein and drugs

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Keywords: G-quadruplex, anticancer drugs, physical chemistry

In the last decades, the discovery of non-canonical nucleic acid structures, such as G-quadruplex (G4), has shed light on a new role for DNA in biology. G4s are formed by guanine-rich sequences that build-up into guanine tetrads stabilized by Hoogsteen hydrogen bonds and monovalent cations. In particular, G4 structures may form *in vivo* in important genomic regions and intervene in several biological processes, including the modulation of oncogenes expression, and therefore represent potential anticancer drug targets [1]. For these reasons, in recent years, the interest has grown in understanding more deeply the energetics of the conformational transitions of these structures [2]. Moreover, non-canonical DNA structures in oncogene promoters have been considered as potential new targets for anticancer therapies, relying on the idea that the overexpression of oncogenes containing these structures can be modulated by specific ligands *in vivo* [3]. In addition, G4-unwinding helicases counter telomere/gene modulation by G4s and are sensibly overexpressed in several cancers. In particular, numerous helicases have been shown to target and regulate G4 structures, and hence play a key role in G4 metabolism [4]. G4-helicase complexes are therefore attractive anticancer targets, and this area significantly overlaps the general field of G4-binders. Here, physicochemical methodologies have been applied to gain information on the energetics of non-canonical DNA structures and their interaction with new putative drugs [5,6] or helicases [7].

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SpyTag/SpyCatcher system: towards an artificial redox biocatalyst

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Keywords: Bioinorganic chemistry, Protein design, Bioinspired catalysts

SpyTag/SpyCatcher (ST/SC) system is a biotechnology tool constituted by an independent oligopeptide (ST) that spontaneously binds covalently to a protein scaffold (SC) through an isopeptide bond, i.e. a secondary amide bond given by the condensation between a carboxyl and an aminic group, standing in the side chain of two different α-aminoacids [1]. This technology comes from a protein called FbaB from *Streptococcus pyogenes* (Spy), a Gram-positive bacterium: it was noticed that after splitting a 13-residues unfolded peptide from the rest of the protein, the two parts spontaneously reconstitute through a covalent isopeptide bond [2]. The protein environment plays a key role in the adduct formation, since it brings residues into optimal orientation and proximity and only some minutes are needed to form the ST/SC adduct [1,2].
The ST/SC system can be exploited for designing new bioinspired redox catalysts; in fact, ST can be designed to interact with the heme group, which is responsible for the redox catalytic activity, interacting, for example, with dioxygen species, and SC is a macromolecule which gives selectivity to the whole catalytic system. The key point of the action mechanism of this tool is that the ST/SC adduct formation provides the anchoring of a metal cofactor, which previously binds to ST, to a protein scaffold, SC.
Heme group is a complex formed by an iron ion (either in its oxidized or reduced form) and a macrocyclic ligand containing four nitrogen atoms, called porphyrin. It is present in the human body both in free form, with a signaling role, and bound to proteins, as metal cofactor; in this case its functions are mainly due to the redox properties of the iron ion. The aim of this work is to study the interaction of the ST peptide with the heme group and the redox reactivity of their complex towards reactive oxygen species (namely, peroxidase-like activity on phenolic and catechol substrates).
The ST peptide sequence, Ac-AHHVPTIVMVDAYKRYK-NH_2, contains the consensus fragment IVMVD, that allows the recognition between ST and SC, and two histidine residues in position 2 and 3, which represent the anchoring site for the heme group (Figure 1).
Firstly, we performed spectrophotometric titrations to estimate binding constants and kinetic studies to evaluate how the presence of ST in solution affects the redox reactivity of the heme group. This study represents the first step of the work, with the aim to investigate also the interaction and reactivity after the isopeptide bond with SC is formed and compare them, to understand whether the presence of the protein could play a role in the redox catalytic properties of the heme-ST system.

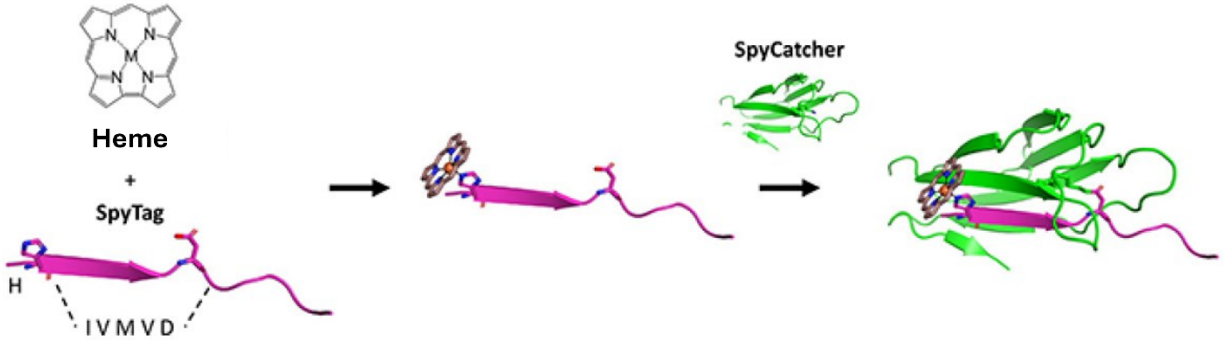

Figure 1. Scheme of the formation of a new bioinspired redox catalyst based on the ST/SC technology.

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Biocompatible nanovectors for treating prostate cancer

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Keywords: Prostate cancer, nanovectors, PLGA-PEG, drug delivery, Angiogenin

Nowadays, prostate cancer (PC) represents the leading cause of cancer-related mortality in men. The clinic profile of patients affected by PC varies based on the stage at diagnosis, highlighting the importance of screenings and accurate detection. Notably, in contrast to the favourable prognosis associated with localized tumours in their early stages of progression, patients with late-stage or metastasized tumours have a very poor prognosis at five years. So far, the treatment of advanced-stage PC involves combined therapy, which usually includes hormone therapy, chemotherapy, and/or radiation [1]. In cases of quite advanced disease, chemotherapy can be combined with anti-androgen therapy, with surgical or chemical castration sometimes employed to reduce cancer cells. Additionally, only a limited number of drugs have been approved by the FDA for the treatment of PC, including taxanes (docetaxel, paclitaxel, and cabazitaxel) [2]. The latter is demonstrated to confer significant advantages when combined with cisplatin analogues. Considering the urgent need for novel strategies, the identification of PC biomarkers has become increasingly crucial. It is widely reported that Angiogenin (Ang), a potent pro-angiogenic protein member of the pancreatic-like ribonuclease superfamily, is a key factor in the progression and invasion of PC. The expression of Ang is observed to increase progressively as prostatic epithelial cells evolve from a benign phenotype to an invasive phenotype [3]. Based on these findings, PLGA-PEG fluorescent biocompatible nanovectors (NVs) loaded with docetaxel, a cisplatin derivative, and a mixture of them were developed. To improve targeting to PC cells, another set of PLGA-PEG NVs decorated with Ang-mimicking peptides was loaded with the same drugs. This results in two effects: impairment of cancer cell angiogenesis and enhanced drug delivery to the tumour site. The physical characteristics of these two sets were evaluated through dynamic light scattering (DLS) and transmission electron microscopy (TEM) techniques. Furthermore, the encapsulated efficiency (EE) of each drug was analysed by HPLC, demonstrating an EE in a range of 8–19% with a concentration of around 10^{-5} M for each pharmaceutical species. In this manner, the decorated NVs can reduce/abolish the intake of the plasma-circulating Ang into the PC cells, and in turn angiogenesis, thanks to the peptidic fragment competing with Ang for the cell-recognition site. This stealth delivery system can potentially enhance drug intake and therapeutic effects, making the drug cocktails suitable even in the case of resistance. Moreover, it enables the drugs' tracking and improves the tolerability of the chemotherapy treatment itself while minimizing off-target reactions. Finally, these two sets of NVs will be tested on PC3 to evaluate their anti-cancer activity.

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The Italian Ministry of University (MUR) is acknowledged for funding the project PRIN 2022- "Biocompatible nanostructures for the chemotherapy treatment of prostate cancer" (2022ALJRPL).

Exploring Kombucha Fermentation Using NMR and HPLC-PDA Analysis

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Keywords: Kombucha, Fermentation, High-Field NMR, HPLC-PDA, Benchtop NMR

Kombucha is a sweetened tea fermented with a symbiotic culture of yeasts and bacteria. The beverage present a balanced taste among sweet, due to the residual sugars, and sour, due to organic acids produced by acetic acid bacteria. In order to evaluate the quality and production reproducibility of kombucha produced through a natural fermentation process an NMR metabolomics approach was applied. Kombucha samples were collected every 5 days for 25 days of fermentation and analysed by 1H-NMR untargeted analysis. The levels of sugars (sucrose, glucose, fructose, trehalose, 2-O-β-Larabinopyranosyl-myo-inositol), organic acids (acetic acid, lactic acid, succinic acid, malic acid, citric acid, formic acid, quinic acid, fumaric acid and gluconic acid), amino acids (alanine, isoleucine, leucine, valine, glutamate, pyro-glutamate, aspartate, and GABA) ethanol, glycerol, caffeine, theanine and gallic acid were quantified. A Benchtop NMR instrument was also used monitoring the quantity of sucrose, lactic acid, acetic acid and succinic acid. The PLS-R statistical analysis with the Benchtop NMR quantitative data was performed obtaining a good prediction of the fermentation stage of kombucha and an indication about its quality and production reproducibility. Finally, the Benchtop NMR spectrometer was demonstrated to be an useful on-line process monitoring tool in the kombucha production. Moreover, this fast analysis method could be applied on different kombucha industrial productions around the world to expand the knowledge about the dynamics of the substrates consumption and metabolites production.

Acknowledgements:
Funder: Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU;
Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods".

Enhancing the specificity of CRISPR-based Sensing Platform via Triplex DNA Probe

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Keywords: CRISPR-Cas, molecular diagnostic, DNA nanotechnology

The extensive use of CRISPR technology for diagnostic applications has been driven by the discovery of the collateral a-specific cleavage activities of CRISPR type V (Cas12) and type VI (Cas13) systems induced by DNA/RNA binding. In particular, Cas12 is a RNA-guided enzyme that integrate single- and double-stranded DNA target recognition, with signal amplification in one system [1]. The collateral cleavage is activated upon binding and is responsible for signal generation, for example, by taking advantage of the Cas12-mediated digestion of single-stranded DNA probes functionalized with a fluorophore-quencher used as fluorescence reporters [2].

Here, we describe a strategy for controlling Cas12a cleavage activity by using a rationally designed DNA-based hybridization network based on the formation of Clamp Triplex DNA. Clamp Triplex are DNA probes that can recognize homopurine DNA/RNA target with superior specificity and affinity compared to standard linear or hairpin DNA probe. When a ssDNA target is present, the Clamp Triplex probe alters its conformation that is associated to a reaction network leading to Cas12a activation and fluorescence output. Our molecular design allows us to overcome two hurdles that limit the application of CRISPR-Cas systems in diagnostics: we are able to distinguish with high specificity a single mutation base on the target sequence comprised between 10 and 20 nt by maintain the same LOD of standard CRISPR-Cas12-based detection systems.

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Spectral Profiling of Extracellular Vesicles Using Mid-IR Spectroscopy and Plasmonic Nanostructures: Potential Applications in Cancer Detection

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Keywords: Extracellular Vesicles; Infrared Spectroscopy; Plasmonic Nanostructures; Hepatocellular Carcinoma; Cancer Detection

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, with cirrhosis identified as a significant risk factor [1]. Traditional blood markers, such as alpha-fetoprotein (AFP), often display poor capability to differentiate HCC from cirrhosis, underscoring the need for more specific circulating markers [2]. In this context, extracellular vesicles (EVs) have shown significant promise, but their translation into diagnostics is hindered by the lack of effective label-free methods for profiling their molecular content [3], [4]. This scenario is further complicated by challenges in purifying and analyzing clinically relevant EV samples, while simultaneously eliminating contaminants. This study explored the potential of mid-infrared (mid-IR) spectroscopy, alone and in combination with plasmonic nanostructures, to distinguish between HCC patients and cirrhotic controls based on the spectral response of EV-enriched samples derived from patients' plasma. To this end, we first utilized conventional mid-IR spectroscopy in the Attenuated Total Reflection (ATR) mode to select the most informative spectral features for patient classification, as in our previous work [5], highlighting significant alterations in the Amide I and II bands of proteins (1475-1700 cm^{-1}). Notably, this spectral signature shows diagnostic performance comparable to AFP (ROC- AUC=0.75) and outperforms it when the two markers are combined (ROC-AUC=0.89). Guided by ATR experiments, the spectral response of samples was further investigated by utilizing a double-resonant plasmonic metasurface suitable for ultrasensitive mid-IR spectroscopy in the 1475-1700 cm-1 range [6]. Specifically, the metasurface consists of two sets of parallel rod-shaped gold nanoantennas (NAs) of different lengths, with the long NA designed to produce intense near-field amplification in the Amide I and II bands. In this design, the short NA is utilized to obtain a sharp reflectivity edge in the 1800–2200 cm-1 range, which is used for EV mass-sensing. Additionally, the use of this nanostructure has allowed us to measure a specific subpopulation of vesicles presenting cancer-related antigens. This was achieved by conjugating NAs with an antibody specific to EpCAM, which is overexpressed in cancer. Employing this approach, we revealed variations in the quantity of EpCAM-presenting vesicles between HCC patients and controls, also uncovering subtle changes in the shape of the Amide I and II regions.

Taken together, the results presented here highlight the potential of mid-IR spectroscopy combined with plasmonic nanostructures as a non-invasive diagnostic tool for the early detection of HCC, based on the spectral characterization of patients' circulating EVs.

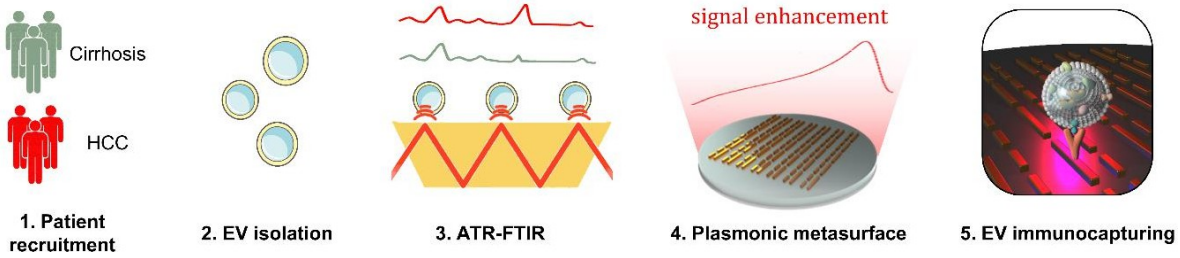

Figura 1:. Experimental setup proposed in the current study. Patient recruitment involved cirrhotic and HCC patients, followed by isolation of circulating EVs. Mid-IR region selection was performed using ATR-FTIR, with signal enhancement achieved via a plasmonic metasurface for ultrasensitive spectroscopy. Finally, extracellular vesicle immunocapturing was conducted to identify spectral markers of cancer-related EVs.

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AMYC BIOMED 2024

Exploring Primaquine Derivatives and their Copper Complexes in Multifactorial Disease Contexts

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Keywords: Primaquine, lactobionic acid, copper.

Primaquine is an 8-aminoquinoline antimalarial agent effective as a tissue schizonticide against intrahepatic forms of all malaria parasites. It is utilized for radical cure of Plasmodium vivax and Plasmodium ovale malaria.[1] Apart from its well-established use in treating malaria, primaquine has attracted attention for its potential applications in addressing various medical conditions.[3] We aimed to explore the biological properties of copper complexes of PQ and its conjugate with lactobionic acid (LA), a disaccharide recognized by the asialoglycoprotein receptor-1 (ASGR1) expressed in hepatocytes [2] that could increase the selective cellular uptake. LA enhances the solubility of PQ and its copper complexes. The conjugate (LAPQ) and its copper complexes species were fully characterized by Mass spectrometry, UV-Vis and Circular dichroism. We found that PQ and LAPQ do not significantly bind zinc ions, which often compete with copper in biological systems. So, these systems are selective for copper ions. The MTT assay was conducted to evaluate the antiproliferative activity of PQ and LAPQ on some human cancer cell lines. Tests were also performed in the presence of copper to assess its effect in terms of final biological activity. Indeed, it was observed that the presence of copper can enhance the toxicity of certain 8-aminoquinolines towards cancer cells, a phenomenon known as "copper-mediated cytotoxicity."
Furthermore, the interaction of PQ with copper may also impact other biological processes, such as antioxidant activity and free radical formation. For these reasons, the ability of PQ and LAPQ to release copper ions under reducing conditions was evaluated in the presence of glutathione (GSH). The antioxidant activity was assessed using the TEAC assay, while radical formation was analyzed using the ascorbate test for the quinoline-copper complexes. Our findings provide an overview of the PQ and LAPQ potential, paving the way to future developments in targeted therapies against overwhelming multifactorial diseases.

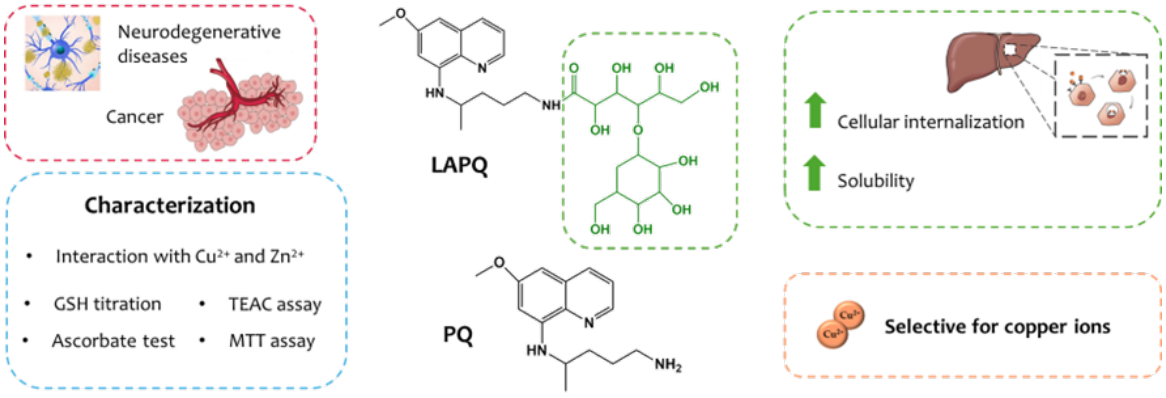

Figure 2 Schematic representation of a novel compound for cancer treatment.

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The impact of polymer coating on nanoparticles interaction with lipid membranes explored by coarse-grained molecular dynamics simulations

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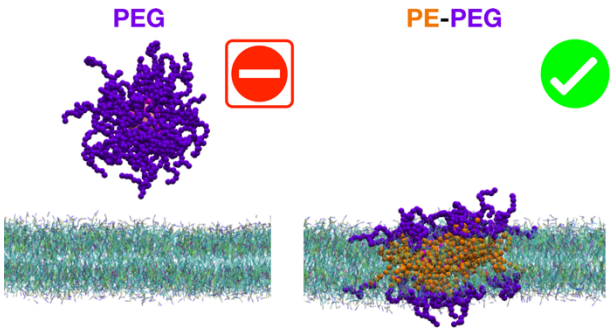
Keywords: Nanoparticles; Polymer coating; Nanomedicine; Lipid membranes; Coarse-grained molecular dynamics

Nanoparticles' (NPs) permeation through cell membranes, whether it occurs *via* passive or active transport, is an essential initial step for their cellular internalization. The NPs' surface coating impacts the way they translocate through the lipid bilayer and the spontaneity of the process. Understanding the molecular details of NPs' interaction with cell membranes allows the design of nanosystems with optimal characteristics for crossing the lipid bilayer: computer simulations are a powerful tool for this purpose. In this study [1], we have performed coarse-grained molecular dynamics simulations and free energy calculations on spherical titanium dioxide NPs conjugated with polymer chains of different chemical compositions. We have demonstrated that the hydrophobic/hydrophilic character of the chains, more than the nature of their terminal group, plays a crucial role in determining the NPs' interaction with the lipid bilayer and the thermodynamic spontaneity of NPs' translocation from water to the membrane. We envision that this computational work will be helpful to the experimental community in terms of the rational design of NPs for efficient cell membrane permeation.



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AMYC BIOMED 2024

Mirror-image glycomimetics: synthesis, biological activity and therapeutic applications

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Keywords: glycomimetics, de novo synthesis, carbohydrate-based route, rare diseases, antibacterial agents

Iminosugars (sugar analogues with an amino function in place of the endocyclic oxygen) represent the most important class of glycomimetics discovered so far [1]. Due to their ability to interact with enzymes involved in key biological functions, these compounds, mainly in their D-enantiomeric form, have been identified as therapeutic candidates for several medical purposes including malignancies, viral infections and rare diseases [2]. Despite their promising pharmacological properties, the progression of these molecules to marketed drugs has been often hampered by their poor *in vivo* selectivity. Conversely, L-iminosugars, non-superimposable mirror images of the natural D-iminosugars, have revealed higher enzymatic selectivity, often acting as inhibitors or enhancers of some glycosidases and glycosyltransferases, and surpassing in some cases the pharmacological potential of their D-enantiomers [3]. However, non-natural L-iminosugars have been less explored compared to their D-counterparts as they are not available from natural sources and few routes have been developed for their chemical synthesis.
Based on these findings, over the last few years our attention has been focused on the preparation of L-*gluco* configured piperidine iminosugar L-DNJ and its alkylated/acylated derivatives (Figure 1) to explore their biological properties for therapeutic applications.

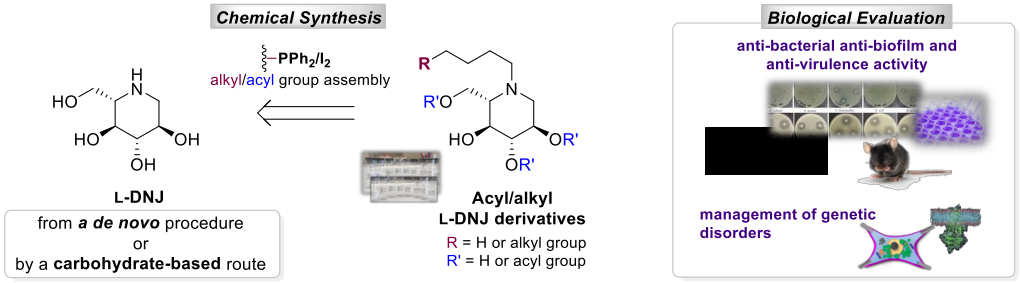

Figure 1. L-DNJ and its alkylated/acylated derivatives: synthesis and therapeutic applications.

Herein we report the synthesis of the enantiomerically pure L-DNJ, achieved by two novel procedures (a *de novo* and a carbohydrate-based route) tuned up in our labs. With L-DNJ in hand, the established PS-TPP/I_2 activating system was exploited for both the assembly of the alkyl/acyl groups and their conjugation with iminosugar core giving access to a small library of L-DNJ derivatives. An overview on the pharmacological properties exhibited by our synthesized compounds is also herein reported. Particularly, *in vitro* and *in vivo* assays revealed their potential in the treatment of some genetic disorders, including Cystic Fibrosis lung disease [4], Pompe [5] and Mucopolysaccharidosis [6] diseases, as well as their ability to act as antibacterial, antibiofilm and anti-virulence agents against multi-drug resistant pathogens [7] pointing out the value of L-iminosugars as novel promising broad-spectrum pharmacological tools.

The study was supported by a financial grant from European Cystic Fibrosis Society (ECFS) and Cystic Fibrosis (CF) Europe (ECFS/CF Europe Post-Doctoral Research Fellowship to A.E.) The work was also supported by Italian Cystic Fibrosis Research Foundation (FFC), grant number: FFC #23/2018, #20/2019, and FFC #13/2020 and by funds from the Cure Sanfilippo Foundation USA 2023 grant "Targeting Heparan Sulfate Proteoglycans as a Novel Therapeutic Strategy for Sanfilippo diseases".

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NMR analysis unveils the atomistic details of the oncogenic binding process between OSCP and IF1

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Keywords: NMR, cancer, structural biology, proteins, structural chemistry

Validating novel targets involved in tumour progression represents a crucial aspect of advancing the field of cancer research. Mitochondrial protein IF1 is overexpressed in many tumours [1], acting as a pro-oncogenic protein but the molecular basis of its tumorigenic role has remained unclear for years. Recently, we found that IF1 protects HeLa cancer cells from the apoptotic death by downregulating the mitochondrial permeability transition pore (PTP) through the binding with the ATP synthase subunit OSCP [2]. The novel protein-protein interaction was first detected in HeLa cancer cells, through immunoprecipitation and proximity ligation assay and then studied in solution by NMR spectroscopy and MS under native conditions. NMR chemical shift perturbations provided the binding epitopes of both protein partners. In detail, a disordered region belonged to IF1 (E29-R39), named pepIF1, weakly interacts with a small surface area of OSCP-NT (A53-I64). We therefore synthesized a peptide with the same sequence of pepIF1 and, thanks to the use of MS under native conditions and NMR, we confirmed that pepIF1 is the minimal sequence required for the binding process. NMR titration was also performed to determine the affinity K_D, that is in the millimolar range, suggesting an ultraweak protein-protein interaction. This allows to the OSCP-IF1 interaction to be efficiently modulated by the cellular levels of IF1, in line with the idea that this binding process is not a physiological event but might mostly occur in tumour masses overexpressing IF1. Finally, we performed Molecular Dynamic Simulations (MDs), guided by NMR, to obtain the atomistic model of the binding complex and we found that pepIF1 is likely folded into alpha helix when it binds the OSCP N-terminal domain.
Our results provide the first completely characterized protein-protein interaction involved in PTP modulation. Moreover, we provide atomistic details about a challenging binding process that is intrinsically difficult to characterize. Our data give useful insights for designing potential anticancer drugs capable of disrupting the OSCP-IF1 oncogenic interaction, thereby removing the protection cancer cells possess against PTP-dependent apoptosis.

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Design, synthesis and biological evaluation of novel *Plasmodium* protein aggregation dyes with multistage antiplasmodial activity

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Keywords: *Plasmodium falciparum*, drug discovery, protein aggregation, YAT2150, bis(styryl)pyrimidinium salts.

Malaria, a disease caused by protozoan parasites of the *Plasmodium* genus, still poses a serious threat to global health. The high mortality rate related to this pathology is expected to increase because of climate change and spreading of the current drug resistance to antimalarial therapies, which include chloroquine (CQ) and its derivatives, atovaquone (AV) and artemisinin (ART)-like drugs, or their combinations [1]. We recently discovered that the bis(styryl)pyrimidinium salt YAT2150, a fluorescent dye used for protein aggregation assays, possesses both the potential to be used for malaria diagnosis and treatment, as it displays strong antiplasmodial activity in all stages of the parasite life cycle [2]. YAT2150 binds to the parasite protein aggregates and interferes with protein aggregation, a phenomenon that seems to be functional for the pathogen and whose impairment could prevent or slow down the onset of drug resistance. The relative high cytotoxicity of YAT2150 on healthy cells and its low selectivity index could hamper its preclinical development. On these grounds, our research group started a lead optimization campaign aimed at the identification of more potent and less cytotoxic YAT2150 derivatives. Three compounds stood out for their improved inhibitory potencies on *P. falciparum* wild type and CQ-, AV-, and ART-resistant strains, low cytotoxicity, selectivity indexes higher than 1000, and gametocytocidal activity in the nanomolar range. The novel derivatives are able to decrease protein aggregation, according to *in vitro* thioflavin-T assays. Moreover, the corresponding pharmacokinetic/pharmacodynamic profiles have been also investigated. Overall, results suggest that this novel class of antiplasmodial compounds represents a striking and innovative alternative for malaria treatment that might advance in the antimalarial drug discovery pipeline.

This work was supported by grants PID2021-128325OB-I00, PID2020-118127RB-I00, and PDC2022-133085-I00, funded by Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación (MCIU/AEI/ 10.13039/501100011033), which included FEDER funds.

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Biochar-based completely paper sensors for bioactive compounds determination in food industry by-products

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Keywords: phenolic compounds; antioxidants; analytical determination; sustainability

The circular economy model promotes the use of sustainable materials and industrial wastes/by-products. In the analytical field, this led to new approaches in which 'recycled materials' are employed as building blocks for analytical device manufacturing. On the other hand, by-products are also sources of bioactive compounds with economic/biological added value that deserve to be analyzed. Among these, phenolic compounds possess nutritional, biological, and technological properties, and their determination through sustainable devices is still an open challenge.
In this work, paper-based electrochemical sensors based on films composed exclusively of nanofibrillar biochar (BH) were manufactured and used for the determination of bioactive compounds in food by-products and derivates; the BH was produced from paper-industry waste, while paper from 100% recycled fibers was used as substrate for the sensors. Nanofibrillar BH was dispersed in water via ultrasound-assisted liquid phase exfoliation, and conductive BH films were formed onto paper and defined using a laser plotter; eventually, the complete paper sensors were assembled via stencil printing. The biochar-based paper sensors (BH-PS) were employed for the analysis of phenolic compounds (i.e., caffeic acid, catechin, chlorogenic acid, and oleuropein) in exhausted coffee, coffee and cocoa bean husk, and artichoke and olive leaf-based supplements. Linear dose-response curves ($R^2 \geq 0.995$) and submicromolar limits of detection enclosed between 0.03 and 0.6 µM were obtained. Moreover, analysis of by-products and derivates returned quantitative and reproducible recoveries (97-114%; RSD≤13%, n=3), consistent with conventional more complex, pollutants, and time-spending analytical methods. Eventually, White Analytical Chemistry principles were used to evaluate the sustainability of the BH-PS-based approach toward traditional analytical methods [1] highlighting economic and environmental advantages; on the other hand, the analytical performance obtained ensures an efficient analysis of phenolic compounds in food by-products and derivatives, allowing to valorize the residual bioactive components.

Figure 1: Sketch of the circularity of biochar-based paper sensors

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Acknowledgements: This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY

Improving Wound Healing assay: achieving High Reproducibility through 3D-Printed Inserts and Automated Cell Analysis

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Keywords: Cell migration, wound healing, 3D insert, biocompatibility, customizable

Massive cell migration and proliferation mechanisms take place in many fundamental physiological processes from embryonic development, to tissue restore and angiogenesis. Damages due to external factors may induce a "wound". Through the wound healing assay, it is possible to observe and investigate the proliferation, migration and behaviour of cells during the "repopulation" of an injury (1). This process is exploited more in general to verify the invasiveness of a cell line or drug effect limiting metastatic processes.
Usually, the wound healing assay is carried out in mechanical way by a tip or a micropipette, to obtain a cell-free region over a cell monolayer. The alternative to the "scratch-wound healing assay" is the "electrical-wound healing" in which the electrical field application removes cells from a specific area of the monolayer. In both cases, cell death, debris accumulation and lack of reproducibility take place as well as difficulty in defining the exact area (2). Moreover, also the image analysis of results can be challenging, despite it is usually performed with open-source programs such as ImageJ. These tools need manual tuning of various parameters, are time-consuming and suffer from limited high-throughput image analysis (3).
To overcome these drawbacks going in parallel toward scratch performance and standardized image analysis, customized 3D-inserts have been developed with a 3D Digital Light Processing (DLP) printer (4) allow a high reproducibility of the assay. We chose a rectangular and round shape of resin inserts. Three cell lines were used to set the test: prostate cancer (PC3), neuroblastoma (SH-Sy5y) and oral cancer (OECM-1), also in presence of TGFβ molecule, a drug able to induce proliferation and migration of cells. In addition, the MATLAB software has been developed to automatically calculate the cell-free area healing, thus overcoming the user-dependent limits of manual border design, especially when the edges became irregular and difficult to be perceived by the human eye.
The aim of MATLAB tool is to realize in one script an algorithm that, collecting a high number of images from different experiments can automatically elaborate all images, detect edges, calculate areas and save all results in one folder. The algorithm is designed to be as much as possible user-friendly, leading the worker to enter only the source and the destination folder.
To conclude, here we would like to show how, thanks to the use of 3D printers and biocompatible resins we are able to realize an ad-hoc device for biological testing. Furthermore, we developed a software that contributes to a growing trend that aims to quickly, accurately and quantitatively analyse high quantities of biological data in automated manner.

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Conjugation and Interaction Studies of Thiol Peptide-based Hydrogels and Peptide Nucleic Acids

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Keywords: Peptide Nucleic Acid, Hydrogels, peptide-based chemistry, Drug delivery.

Peptide Nucleic Acids (PNAs) are synthetic analogues of nucleic acids whose backbones consist of *N*–(2-aminoethyl)glycine repeats, anchored via amidic bonds.[1] The exceptional physicochemical properties, together with the remarkable stability in biological fluids and the extremely low toxicity, have made PNAs among the most valuable nucleic acid mimetics to be employed for smart delivery purposes as well as for the development of new diagnostic tools.[2,3] On the other side, ultrashort aromatic peptide-based multicomponent hydrogels (HGs) have been largely employed as biocompatible matrices for several applications in biotechnology, ranging from tissue engineering, drug delivery, and biosensor production. One of the most explored hydrogelators is the low molecular-weight Fmoc-FF (*N*α-fluorenylmethoxycarbonyl-diphenylalanine) homodimer due to its well-studied gelating properties.[4] From that starting point, the knowledge of Fmoc-FF aggregation properties has been used as a guide to design new peptide-based gelators, with implemented features for the development of smart delivery and/or diagnostic tools.[5] Since hybrid hydrogels are non-toxic, the idea is to employ those matrices as scaffolds for controlled drug release in the presence of a reducing environment, such as the tumour microenvironment.[6] In this regard, the functionalization of mixed (Cys)HG at different molar ratios compared to Fmoc-FF (1/5, 1/10 and 1/20, respectively) with (Cys)PNA molecules via specific and non-specific interactions is shown here, followed by supramolecular characterization through several techniques, such as HPLC, MS, CD, FT-IR, NMR and microscopy.

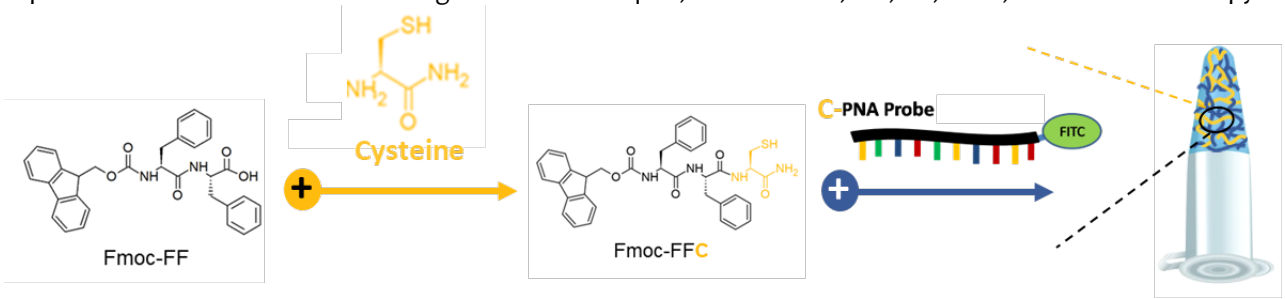

Figure 1. Schematic representation of Fmoc-FFC and C-PNA-FITC probe forming supramolecular hydrogels.

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AMYC BIOMED 2024

Unveiling the interaction between DNA G-quadruplexes and an R/G-rich peptide

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Keywords: G-quadruplexes (G4s), G4-binding peptide, Biophysical characterization.

G-quadruplexes (G4s) are non-canonical secondary structures formed in G-rich DNA and RNA sequences and characterized by the stacking of two or more G-tetrads.[1] G4 structures are implicated in various biological processes and diseases, including cancer, representing a promising target for therapeutic intervention.[1] G4 formation is closely related to the interaction with several proteins that bind and stabilize or unfold them. A recent study revealed a common motif among 77 human G4-binding proteins, consisting of a 20-mer R/G-rich sequence, named NIQI (Novel Interesting Quadruplex Interaction motif).[2] However, its ability to physically interact with G4 structures has never been experimentally demonstrated.

Herein, we synthesized this peptide, and several biophysical techniques were employed to characterize the interaction between the R/G-rich NIQI peptide and various G4 DNA structures. The peptide's secondary structure was predicted using AlphaFold2 Colab and confirmed through Circular Dichroism (CD) experiments. CD-melting experiments were conducted to evaluate the peptide's ability to thermally stabilize different G4 topologies. Microscale thermophoresis (MST) and Isothermal titration calorimetry (ITC) experiments were performed to quantify the binding affinity and stoichiometry of the interaction between NIQI and various G4s and to verify its selectivity for G4s over duplex DNA. Furthermore, Nuclear Magnetic Resonance (NMR) experiments were recorded to understand the binding mode of NIQI identifying the DNA regions affected by peptide binding (Figure 1). Our study highlights the key amino acids involved in the interaction and lays the basis for the development of new peptide-based G-quadruplex ligands, promising alternatives for interfering in DNA-protein interactions and potentially offering therapeutic avenues.[3]

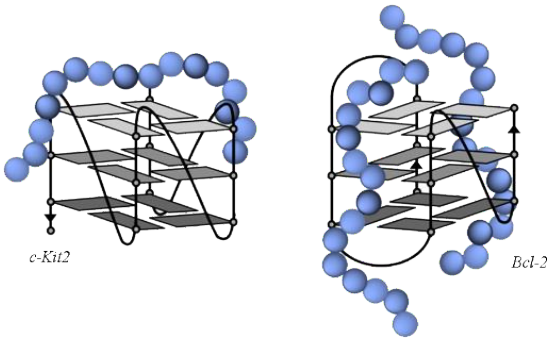

Figure 1: Schematic representation of possible binding modes for the strongest interactions of NIQI peptide (in blue) to *c-Kit2* and *Bcl-2* G4s.

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Enzyme-based electrochemical point-of-care test towards liquid biopsy applications

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Keywords: biosensors, biomarkers, microRNA, cancer, diagnosis

Lung cancer (LC) is one of the most common causes of death worldwide: non-small-cell lung carcinoma (NSCLC), an aggressive LC type, represents about 80% of overall LC cases.[1] Rapid detection of blood-circulating biomarkers through non-invasive liquid biopsy can significantly enhance diagnosis and prognosis, thereby improving survival rates. However, even if we are in the era of personalized care, sophisticated and time-consuming procedures still represent drawbacks for cancer management/intervention.[2] Recently, clinical studies highlighted a correlation between blood-circulating microRNAs (miRNAs), namely miRNA signature, and immune checkpoint therapy response of NSCLC patients that have been highlighted as valuable biomarkers for NSCLC prognosis and therapy-follow up.[3] Taking into consideration these emerging biomarkers within the framework of personalized medicine, affordable and portable devices could facilitate quicker monitoring for cancer patients, including those in remote areas. In this context, we have developed a novel class of point-of-care (POC) test boosted by the use of duplex-specific nuclease (DSN) as the recognition element of miRNA. The DSN enzyme exhibits high selectivity to discriminate DNA-RNA heteroduplexes triggering isothermal target recycling and signal enhancement. This capability is crucial for overcoming the limitations often associated with detecting trace amounts of miRNA in biofluids. In our study, the specific probe for the miRNA target was labelled with a redox mediator, methylene blue, to enable the detection of enzymatic products via electrochemical measurements at screen-printed electrode. Optimization studies have been performed to obtain the optimum enzymatic reaction in terms of probe and enzyme concentration. A calibration graph was obtained for different target concentration ranging from 0-100 pM and a limit of detection down to the fM level was achieved. Specificity studies were performed with various miRNA targets, and finally the device was applied in spiked serum samples. These promising results suggest potential for developing a paper-based strip to realize an all-in-one sustainable platform for liquid biopsy applications.

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The endocannabinoid anandamide activates pro-resolving pathways in human primary macrophages by engaging both CB2 and GPR18 receptors

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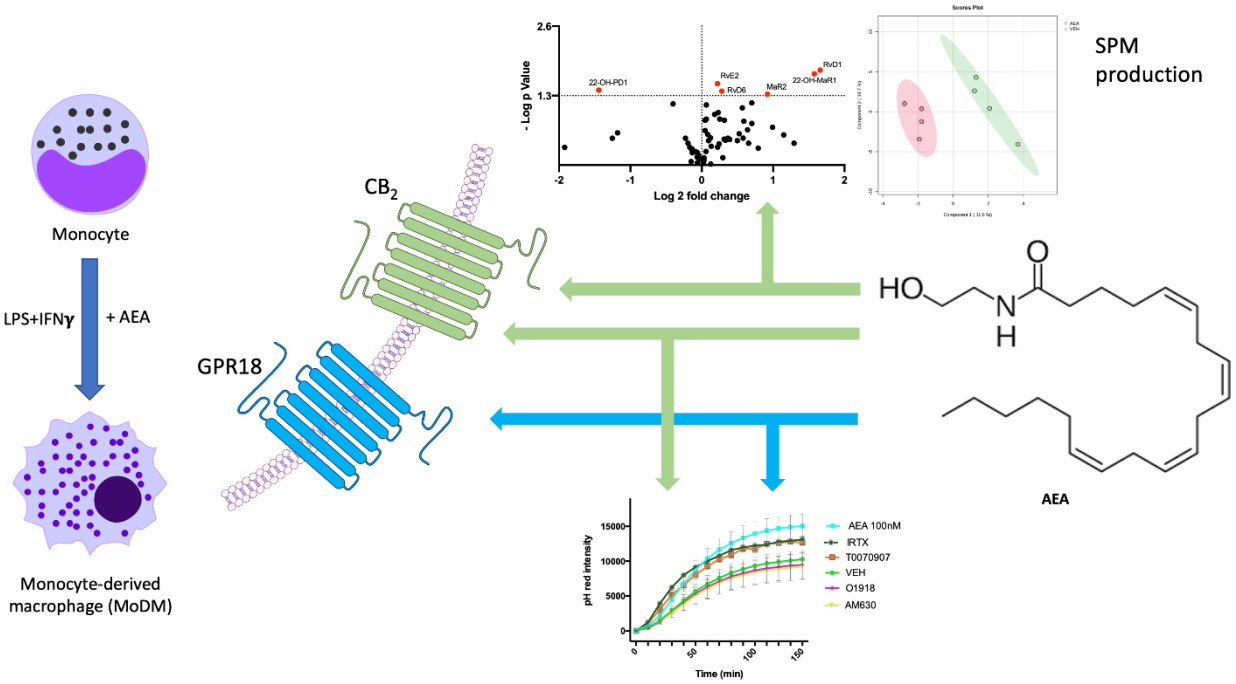
Keywords: endocannabinoids**, r**esolution of inflammation, specialized pro-resolving mediators, CB_2, GPR18.

Resolution of inflammation is the cellular and molecular process that prevents widespread and uncontrolled inflammation, while restoring tissue function in the aftermath of acute immune events[1,2]. This process is orchestrated by specialized pro-resolving mediators (SPM), a class of bioactive lipids that reduce immune activation and promote removal of tissue debris and apoptotic cells by macrophages. Although SPMs are the lipids that have been best studied for their role in facilitating the resolution of self-limited inflammation, a number of other lipid signals, including endocannabinoids, also exert protective immunomodulatory effects on several immune cells, including macrophages. These observations suggest that endocannabinoids may also display pro-resolving-like actions. Interestingly, the endocannabinoid anandamide (AEA) is not only known to bind canonical type 1 and type 2 cannabinoid receptors (CB1 and CB2), but also to engage SPM-binding receptors such as GPR18. This suggests that AEA may also contribute to the governing of resolution processes. In order to interrogate this hypothesis, we investigated the ability of AEA to induce pro-resolving responses by classically-activated primary human monocyte-derived macrophages (MoDM). We found that AEA, at nanomolar concentration, enhances efferocytosis in MoDMs in a CB2- and GPR18-dependent manner. Using lipid mediator profiling we also observed that AEA also modulates SPM profiles in these cells, including levels of Resolvin (Rv)D1, RvD6, Maresin (MaR)2, and RvE1 in a CB2-dependent manner. AEA also modulated expression of SPM enzymes involved in the formation and further metabolism of SPM such as 5-lipoxygenase and 15-Prostaglandin dehydrogenase. Our findings show, for the first time, a direct effect of AEA on pro-resolving pathways in human macrophages and provide new insights into the interaction between different lipid systems controlling pro-resolving responses that reestablish homeostasis in the aftermath of acute inflammation.



N-arachidonoylethanolamine (AEA, or anandamide) is one of the two main endocannabinoid (eCB) lipids, and displays well-described immunomodulatory properties. This lipid is able to bind both type-2 endocannabinoid receptor (CB2) and GPR18 – i.e., the receptor of resolving D2 (RvD2) a specialized pro-resolving mediators (SPM). This suggests an interaction between these two lipid systems in modulating inflammatory events and their resolution. Here we report that AEA induces bona fide pro-resolving properties in human primary monocyte-derived macrophages (MoDM) at nanomolar range; in particular it enhances efferocytosis in a CB2- and GPR18-dependent manner, and is able to profoundly shift the lipidomic profile of MoDMs towards the biosynthesis of pro-resolving lipids through by engaging CB2.

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QM/MM modelling of a natural photoenzyme: structural and mechanistic insights in fatty acid photodecarboxylases

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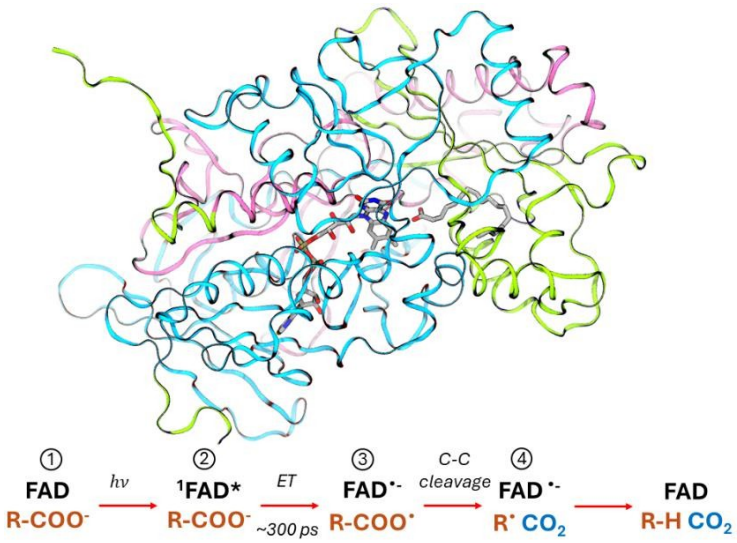
Keywords: Biocatalysis, photoenzymes, fatty acid photodecarboxylases, photochemistry

In photoenzymatic catalysis, a cofactor within the enzyme's active site becomes excited upon light absorption and promotes electron or energy transfer to convert an organic substrate into a given product. Currently, only three natural photoenzymes have been identified, with the most recent discovery being an algae-derived fatty acid photodecarboxylases (FAPs). In FAPs, a cascade of reactions is initiated upon photoexcitation of a flavin adenine dinucleotide (FAD) cofactor. By acquiring a more oxidizing character with respect to its ground-state counterpart, the singlet excited state of the FAD (^{1}FAD*) oxidizes the carboxylate group of a deprotonated free fatty acid (R- COO^-). The resulting electron transfer (ET) occurs in ~300 ps and leads to the formation of two reactive radical species: the anionic semiquinonic ($FAD^{\bullet-}$) and the R-$COO^\bullet$ species. Upon ET, the latter is quasi-instantaneously prone to decarboxylation, thereby generating an acyl radical intermediate ($R^\bullet$) and releasing CO_2 in the active site. Irrespectively of the mechanisms suggested in the literature [1,2], the catalytic cycle is completed with the restoration of the oxidized FAD cofactor and the formation of the alkane R-H, a product of interest for chemicals, pharmaceuticals, cosmetics, and biofuels.
In this contribution we provide new insights into the structural and mechanistic features that promotes both the ET from the fatty acid substrate to the excited FAD and the decarboxylation process. By means of hybrid quantum mechanics / molecular mechanics (QM/MM) strategies, on the one hand we unravel the complex excited-state energy landscape of the FAD cofactor, thereby understanding the driving force for the ET to occur and modelling this process through the classical Marcus theory. On the other hand, polarizable embedding QM/MM dynamics allow us to characterize the decarboxylation process, occurring in tens of ps with a very low energetic barrier.



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The continued threat of emerging and re-emerging flaviviruses: *in-silico* approaches applied to studying potential NS3 binders

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Keywords: Flaviviruses, NS3, helicase, protease, CADD

Nowadays, zoonosis caused by Flaviviruses represents an established ongoing threat to global health. To date, no antiviral treatments are available [1]. The *Flaviviridae* family members comprise many enveloped viruses, including Dengue (DENV, isoforms 1-4), West Nile, and Zika. These small spherical particles incorporate a single genomic RNA of positive-sense polarity encoding three structural and seven non-structural proteins (NS) [2-3]. The numerous crystal structures of the enzymatic NS proteins available in the Protein Data Bank (PDB) allow us to accelerate further molecular understanding and improve the structure-guided drug discovery process.

In this panorama, the NS3 complex (protease and helicase) is essential for viral replication and represents a valid druggable target. Combining classical and advanced computational methods, such as Structure- and Ligand-based approach, Virtual Screening (VS), conventional and accelerated Molecular Dynamics simulations (MDs), and Dynophore, we aim to identify new potential inhibitors able to bind different sites (catalytic or allosteric), thus altering the conformational equilibrium of the protein function. This approach may prove worthwhile in developing new broad-spectrum inhibitors for DENV, WNV, and ZIK viruses.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no.PE00000007, INF-ACT)

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A Multi-Use Conductive Ink Formulation for Enzyme-BasedFlexible and Wearable Bioelectronics

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Keywords: conductive ink, amperometric biosensor, wearable biosensor, glucose detection.

Developing disposable and low-cost electrochemical devices for biomedical applications has significantly increasedwith the diffusion of remote diagnostics. This is probably due to the need for regeneration of the conventionalsensor surfaces and the demand for production processes that allow the manufacture of disposable and portableelectrochemical devices promoting the reduction of the volume of samples, in-situ detections, and lower cost. [1] Conductive inks are used for the development of disposable electrochemical sensors. They trigger the possibility of building screen- or stencil-printed electrodes with similar efficiency with respect to solid electrodes. [2] In particular, biocompatible inks are formulated and stencil-printed on a flexible support that could be easily integrated withinsmart-devices for the continuous and minimally invasive monitoring of lactate and glucose, as shown in figure 1. First, the conductive ink formulation has been optimized based on electrochemical and rheological measurementsimplemented within a multivariate analysis model. Afterwards, the active carbon electrode was modified with osmium redox polymers (ORPs) to establish an electronic connection with enzymes, since neither glucose oxidase(GOx) nor lactate oxidase (LOx) are able to directly transfer electrons. [3,4] Finally, both biosensors have been tested in model solution and sweat to determine the analytical figures of merit (e.g., LOD, LOQ, linear range, sensitivity, selectivity, reproducibility, stability, storability etc.).

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Detecting microRNAs as Laryngeal Cancer biomarkers via SERS biosensor based on Electrospun Nanofibers decorated with in situ synthesized Gold Nanoparticles.

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Keywords: microRNAs, gold nanoparticles, electrospun nanofibers, optical biosensor, SERS

Laryngeal Cancer (LCa) represents one-third of all head and neck cancers and is characterized by high mortality and morbidity due to late diagnosis. microRNAs (miRNAs) are small non-coding RNAs (18-20 nucleotides) involved in post-transcriptional gene regulation that are emerging as potential biomarkers of several cancers, including LCa [1] MiRNAs are present in different body fluids (such as blood, plasma, saliva, sweat, and so on) at very low concentrations, down to femtomolar (10^{-15}M) level, hence it is challenging to detect miRNAs using the old standard molecular techniques (PCR, Northern blot, Microarrays and so on). In recent years, nanotechnology has provided new diagnostic and prognostic strategies for miRNA detection by offering the possibility of constructing nanostructured biosensors [2]. Here, we present a Surface Enhanced Raman Spectroscopy (SERS) [3] biosensor for detecting microRNAs as LCa biomarkers. The proposed biosensor consists of *in situ* synthesized Gold Nanoparticles on electrospun polymeric nanofibers. An amino-terminal molecular beacon DNA probe labeled with Cyanine 3, as Raman Reporter, allows a specific interaction with target miRNA 223-3p, resulting to be overexpressed in affected patients. This label and enzyme-free optical approach enables an easy, sensitive, and cost-effective miRNA detection, resulting in a Limit of Detection (LOD) of femtomolar (fM) level. The binding specificity was further tested using a non-complementary miRNA sequence, showing any change in the SERS signal. Results in the characterization and application of this system will be discussed.

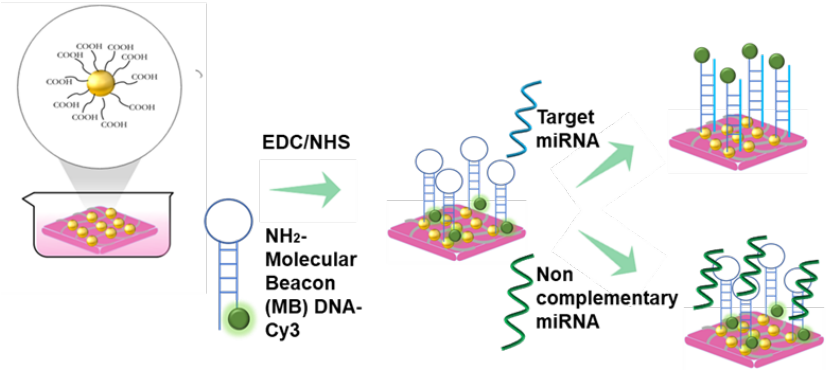

Figure 1. SERS biosensor based on Electrospun Nanofibers decorated with in situ synthesized Gold Nanoparticles.

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Comprehensive characterisation of *Taraxacum officinale* phytochemical profile and neuroprotective properties

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Keywords: dandelion, NMR, HPLC-DAD, polyphenols, neuroinflammation

Officinal plants are a source of secondary metabolites with biologically relevant properties [1]. Due to the variability in the production of these secondary metabolites caused by multiple factors (genetics, light, temperature, humidity, altitude, soil nature and cultivation), it is necessary to assess the difference between ecotypes of the same species. The primary objective of this study is to characterise the chemical profile of dandelion's three different ecotypes (spontaneous, land and mountain vs. organic ecotypes). By detecting possible differences between the ecotypes of the same cultivar, we aim to significantly contribute to understanding the variability in the production of secondary metabolites, a crucial and significant area of research in plant biology and pharmacology.
The dandelion is entirely edible and used for culinary purposes and in the food industry, making it nutrient-rich. *Taraxacum officinale* has been extensively studied for its biological properties, but the literature lacks information on the neuroprotection activity.
All samples were subjected to the Bligh-Dyer extraction protocol [2] to obtain extracts for metabolomic analysis using NMR spectroscopy. The matrices were extracted following a green extraction protocol to perform HPLC-DAD analysis, identifying and quantifying polyphenols, and obtaining extracts to carry out biological assays.
Different classes of compounds, such as sugars, organic acids, free amino acids, polyphenols, fatty acids and other compounds from NMR analysis were identified and quantified. By comparing the variability of each metabolite, some were tissue-specific markers: arginine was found in the roots, whereas DGDG, pheophytin and chlorophyll were identified in the aerial parts. The concentration of metabolites varied significantly depending on the culture, tissue type and ecotype. The mountain ecotype had the wealthiest phytochemical profile because of specific polyphenols, caftaric acid and chicoric acid. Also, by targeted chromatographic analysis, the mountain dandelion was the ecotype with the highest concentration in some phytochemicals, including chicoric, caftaric, chlorogenic, and caffeic acids.
In addition, the mountain ecotype hydroalcoholic extracts were tested on HypoE22 cells employing MTT to assess biocompatibility and antioxidant activity. The same extracts were tested on mice brain specimens to evaluate the neuroprotective effect through RT-PCR of some genes. The results obtained from the biological assay indicated that the extracts, based on their phytochemical content, could reduce neuroinflammation.
The dandelion showed considerable potential for bioactivity, as the identified molecules are well known for their antioxidant action, activity in modulating the body's metabolic biochemical processes, and pronounced anti-inflammatory effect. All this information provided can be used to support research in the nutraceutical and phytopharmaceutical industry.

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Mucoadhesive electrospun systems for the oral cancer treatment

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Keywords: *fibers, biopolymers, electrospinning technique, oral cancer.*

Oral cavity cancer is one of the most common malignant tumors [1]. The standard treatment is surgical resection, accompanied or replaced by chemo and radiotherapy. However, this approach is quite invasive, and patients are often diagnosed at an advanced stage. Therefore, it is necessary to propose valid alternatives, promoting a pharmacological strategy. Traditional treatments for oral cancer are based on corticosteroids and dermatological formulations. This approach has many disadvantages, such as taste alteration, difficulty of application, and reduced efficacy. To address this problem, drug delivery systems have been developed to act locally, reducing the numerous side effects of the drug. These systems offer several advantages aimed at improving the effectiveness of the active ingredient by allowing prolonged release over time. The characteristics that the carrier must possess are biodegradability, biocompatibility, and sustained release. In this context, the present work proposes an innovativedrug delivery system, based on fibrous membranes, for the treatment of oral cavity cancer, acting directly *in situ* and reflecting the characteristics above described. In particular, polylactic acid (PLA), a biodegradable, biocompatible polyester with low carcinogenicity, was chosen to produce fibers loaded with clobetasol and dexamethasone for the treatment of oral cancer in the biomedical field [2]. The obtained fibrous mats were coated with chitosan, a low-cost natural polysaccharide, to provide mucoadhesive properties.

The structure of the PLA fibres was observed at the SEM, evidencing the obtainment of uniform, randomly oriented, defect free fibres. For chitosan-coated fibres, the deposition of a homogenous coating is confirmed. Furthermore, the successful deposition of the chitosan coating was confirmed by measuring the contact angles of the PLA fibres. Direct and indirect cytotoxicity and MTT tests were carried out on the samples to assess compatibility with the physiological system: the results obtained were optimal, as the mortality index was below 30%, a useful condition to define a non-toxic material. In addition, the fibres produced were subjected to kinetic release tests to assess thevalidity of these systems: what could be deduced was that the chitosan-coated fibres had released 56% clobetasol and 65% dexamethasone at 1h compared to 92% released by the fibres without resurfacing. This result was encouraging as it represents an optimal situation for the development of drug delivery systems.

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Development of an enzyme-based screen-printed biosensor integrated into a 3D printed wearable device for sweat lactate monitoring

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Keywords: enzyme-based screen printed biosensors, lactate, wearable, 3D printing, healthcare

Lactate detection plays a crucial role in various fields, including healthcare, sports science, and food industries. In particular, real-time and continuous monitoring of lactate levels in sweat is used as an indicator of physiological information to evaluate exercise outcomes and sports performance [1]. Considering that conventional methods for lactate determination often lack the sensitivity, portability, and ease of use needed for on-body applications, electrochemical biosensing is emerging as the most widely used approach for lactate determination. In fact, wearable bioelectronic devices offer a solution by integrating lactate-specific enzymes with electrochemical transducers, enabling real-time, non-invasive monitoring of this important analyte [2]. In this work, the development of a wearable lactate biosensor, obtained through the modification of homemade screen-printed electrodes with a bio-hybrid probe which included prussian blue, carbon black, and lactate oxidase enzyme, was carried out. After the optimization of several parameters, such as the optimal enzyme amount to be immobilized onto the modified electrode surface and the reaction time between the enzyme and the substrate, the system was tested at increasing levels of lactate in buffer solution and in 1% sweat, achieving a good linearity up to 10 mM and a satisfactory repeatability of 5%. The developed lactate chronoamperometric monitoring system was validated against a standard laboratory technique on three different sweat samples, obtaining a strong correlation (94 - 105%) with LC-MS/MS standard method. To enable wearable integration, the lactate biosensor can be incorporated into a wearable 3D printed device designed for sweat collection and analysis. The device features a sweat inlet, a sample chamber, and a biosensor integration platform. The 3D printed design allows for customizable and conformal fit to the user's skin, ensuring efficient sweat collection and transport to the sensor. This work contributes to the advancement of non-invasive, continuous, and personalized physiological monitoring through the integration of screen-printed biosensors into 3D printed wearable devices.

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Spectral Examination of Blood Components in Diabetic Patients with Neuropathic Complications: Assessing the Diagnostic Potential of Extracellular Vesicles

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Keywords: spectroscopy, diabetes, vesicles, neuropathy

Diabetic sensorimotor peripheral neuropathy (DSPN) is the most prevalent neuropathic complication of diabetes mellitus (DM), significantly impacting the patient's quality of life[1,2]. Early intervention is key to slowing down the progression of DSPN, underscoring the importance of a timely diagnosis, currently based on patients' medical history and physical examinations[3]. In this study, we aim to identify novel markers of DSPN in DM patients by investigating several blood components, including plasma, red blood cells (RBCs), RBC-derived ghost cells, and extracellular vesicles (EVs).

To achieve our objectives, we employed Fourier-transform infrared (FTIR) spectroscopy, a fast and cost-effective method that provides unique spectral fingerprints of biological samples. We collected samples from 72 patients with type 1 diabetes (T1DM), of whom 50 did not exhibit neuropathy and 22 were diagnosed with DSPN. Notably, we found statistically significant differences in the spectral signatures of extracellular vesicle (EV) samples between the two groups, specifically in the amide I and II, lipid ester, and CH-stretching bands. Moreover, we developed a combined marker using logistic regression and ROC analysis, which integrated the intensity of the amide I peak with the duration of the disease. This marker showed a strong ability to identify diabetic patients with DSPN, with an AUC of 0.88 and a 95% confidence interval of 0.74-1.

This research highlights potential blood-based markers for DSPN through the use of FTIR spectroscopy.

We identified notable differences in the spectral signatures, especially in EV samples. The combined marker we developed, which incorporates the intensity of the amide I peak and the duration of the disease, demonstrates strong discriminatory capabilities. This suggests that this approach could be effectively used to create new screening methods for diabetic neuropathic complications.

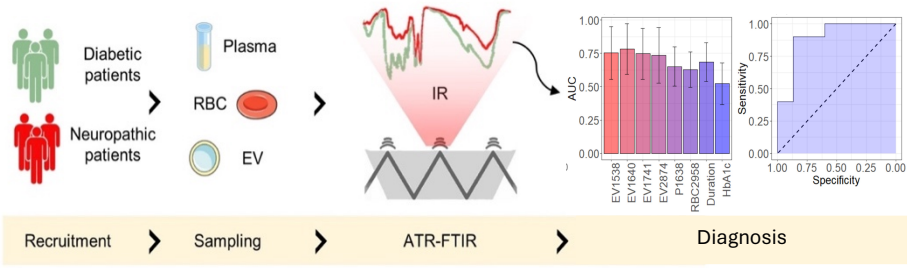

Figure: Experimental design

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Carbohydrate-modified Hydrosilylated Porous Silicon Nanoparticles via one-pot Passivation-Functionalization Chemical Strategy for Biomedical Applications

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Keywords: Porous Silicon Nanoparticles, Carbohydrate-modified nanoparticles, passivation, hydrosilylation

In recent decades, nanotechnology has emerged as a transformative strength within diagnostic and therapeutic sciences, heralding novel paradigms in diagnosis, treatment, and targeted oncological interventions. Compared to conventional chemotherapy, characterized by its non-selective eradication of proliferating cells and attendant harmful side effects, nanotechnological modalities promise a nuanced and precise approach to cancer management.

Among nanotechnological methodologies, systems predicated upon porous silicon nanoparticles (PSiNPs) have emerged as promising due to their capacity for precise tumor cell targeting while concurrently mitigating off-target effects. However, antecedent approaches to PSiNPs functionalization have been burdened by inherent complexities, notably their incompatibility with thermolabile compounds.

Our research sought to pioneer a streamlined and productive protocol for PSiNPs functionalization via hydrosilylation in response to these difficulties. This novel approach integrates mild temperature conditions and the judicious utilization of a Lewis acid catalyst, thereby circumventing the challenges associated with heat-sensitive molecules. Specifically, our focus was on the conjugation of PSiNPs with Allyl-tetra-O-acetyl-β-D-glucopyranoside (Al-s), a carbohydrate moiety characterized by an allyl functional group.

Empirical validation of our methodology, ascertained through rigorous thermogravimetric analysis, underscored its efficacy in conferring robust protection upon PSiNPs against degradation. Moreover, the salience of this innovative hydrosilylation protocol extends beyond mere technological refinement, heralding transformative prospects within the domain of brain-targeted drug delivery. Notably, our methodology holds promise in the selective targeting of brain cancer cells exhibiting overexpression of GLUT receptors, thereby engendering a paradigm shift towards bespoke and efficacious therapeutic interventions.

In summation, the convergence of PSiNPs-based nanotechnology with our pioneering hydrosilylation protocol represents a seminal milestone in the annals of oncological therapeutics, heralding a future wherein tailored pharmacotherapeutic agents can be delivered with pinpoint accuracy to diseased tissues, thereby minimizing systemic toxicity and maximizing therapeutic efficacy.

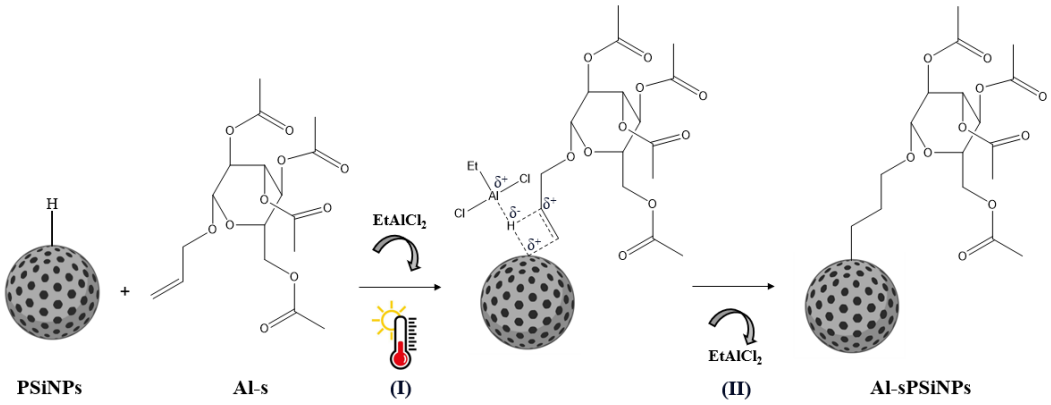

Figure 1. Schematic representation of the Hydrosilylation of PSiNPs; (I) Al-s, in the presence of $EtAlCl_2$ (as a catalyst) and of mild-high temperature, reacted with PSiNPs surface achieving a reaction intermediate with the catalyst that then (II) led to the reaction product, Al-sPSiNPs.

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Rational identification of new STING agonists with broad-spectrum antiviral activity

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Keywords: STING, virtual high throughput screening (vHTS), broad-spectrum antiviral, immune response

The Stimulator of Interferon Genes (hSTING) plays a crucial role in the immune responses by mediating the cGAS-STING signalling pathway [1]. The enzyme cGAS (cGAMP synthase) detects cytoplasmic DNA resulting from cellular damage or viral infections, producing 2'-3'-cGAMP, which activates STING [2].
This activation triggers type I interferon production (IFN-I), making STING a promising target for broad-spectrum antiviral drug development [3]. To identify new potential STING agonists, a ligand-based virtual high throughput screening (vHTS) was carried out using known STING agonists as queries. The databases (DBs) screened include commercial and in-house DBs containing synthetic and natural compounds, which led to the identification of eight initial candidates: six natural and two synthetic molecules. Biological assays were performed to evaluate the ability of the compounds to induce IFN-I production via STING activation, revealing synthetic compounds featuring a benzofuran scaffold as particularly promising. To further investigate the potential of this scaffold, a focused library of 13 benzofuran derivatives was synthesized and evaluated for their ability to act as STING agonists. These compounds were evaluated using a gene reporter assay, which measures luciferase activity driven by the human IFN-β promoter in HEK293T cells expressing exogenous STING. Seven of them were able to induce IFN-β transcription, whereas no induction of the IFN promoter was observed in the presence of a mutated and inactive STING. To evaluate their antiviral activity, BZF derivatives were initially tested in BEAS-2B cells infected with hCoV-229E, and three compounds demonstrated significant inhibition of viral replication with EC_{50} values in the μM range. These findings were further validated in SARS-CoV-2-infected cells, confirming their antiviral activity. To prove their mechanism of action, the compounds were assessed in Vero E6 cells, which lack IFN-I production. As expected, they did not inhibit SARS-CoV-2 replication in these cells, indicating that their antiviral effect is likely mediated through IFN-I induction. In addition, molecular docking experiments were performed using a validated protocol to predict the binding mode and rationalize the biological activity. Docking results provided insights into the binding modes of the synthesized compounds and will guide further optimization efforts.

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Fabrication of Functionalized Polymeric Microneedles for biomedical applications

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Keywords: Microneedles, PLGA, micro-fabrication, pyro-electrohydrodynamic effect

Polymeric Microneedles (MN) possess tunable properties that can be exploited for many applications in the biomedical field. The minimal invasiveness of these systems is a peculiarity that could be used for creating portable devices for continuous health monitoring, diagnostics and therapeutic applications [1-3]. PLGA is a synthetic copolymer with tunable degradation time (from weeks to months) and enough mechanical properties for skin indentation which meets the needs of biodegradability, biocompatibility and low toxicity, so it has been largely studied for MN fabrication [4].
Herein, we investigated the realization of different PLGA MN systems, both in single and in matrix configurations. In particular, the realization of single needles was obtained by an innovative electro-hydrodynamic technique, mediated by a pyro-electric crystal (p-EHD), which showed a spontaneous polarization when thermally stimulated with the consequent generation of an electric field, that drew a sessile drop of the polymeric solution into a conical structure (Figure 1 a) [5-8]. The p-EHD allowed to fast produce microneedles directly and in a single step, through a stamp-less and contact-free process at room temperature. As to the standard micro-molding technique, it was employed for needles' mass production in the array configuration. The morphological characterization of the obtained microstructures was studied with a particular regard to the initial conditions. By the p-EHD conical structures of 500 µm high were fabricated, while using the micro-molded matrix needles of 300 µm and height of 150 µm were obtained. Thanks to our engineered platform, we demonstrated that micro-structures with good shape and optimum mechanical properties were fabricated with a view to using them both for pain-free biosensing and disease therapies.

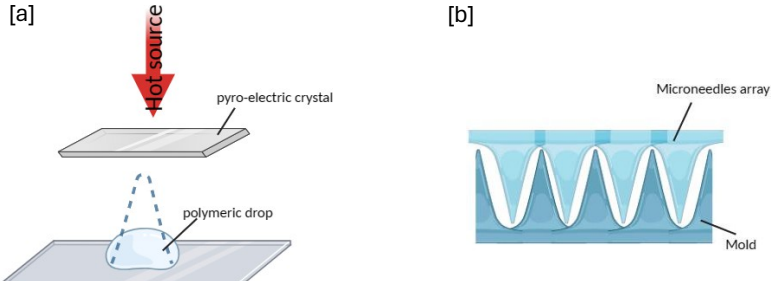

Figure 1 Schematic representation of: (a) the p-EHD process; (b) the micro-molding

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AMYC BIOMED 2024

Multiscale simulations reveal key features of the ultrafast excited-state dynamics of Luteins in the major light-harvesting complex LHCII

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Keywords: light-harvesting complexes (LHC), ultrafast excited-state dynamics, molecular dynamics (MD), quantum mechanics/molecular mechanics calculations (QM/MM), surface hopping simulations (SH).

Carotenoid pigments are known to present a functional versatility when bound to light-harvesting complexes. This versatility originates from a strong correlation between a complex electronic structure and a flexible geometry that is easily tunable by the surrounding protein environment. In this presentation, we show our results on how the different L1 and L2 sites of the major trimeric light-harvesting complex (LHCII) of green plants tune the electronic structure of the two embedded luteins, and how this reflects on their ultrafast dynamics upon excitation. By combining molecular dynamics and quantum mechanics/molecular mechanics calculations, we found that the two luteins feature a different conformation around the second dihedral angle in the lumenal side (Figure 1, top-left). The s-cis preference of the lutein in site L2 allows for a more planar geometry of the π-conjugated backbone, which results in an increased degree of delocalization and a reduced excitation energy, explaining the experimentally observed red shift (Figure 1, top-right). Despite these remarkable differences, according to surface hopping simulations the two luteins present analogous ultrafast dynamics upon excitation: the bright state S_2 quickly decays (in ~50 fs) to the dark intermediate S_x, eventually ending up in the S_1 state (Figure 1, bottom-center). Furthermore, by employing two different theoretical approaches (i.e., Förster theory and an excitonic version of surface hopping), we investigated the experimentally debated energy transfer between the two luteins. With both approaches, no evident energy transfer was observed in the ultrafast timescale.[1]

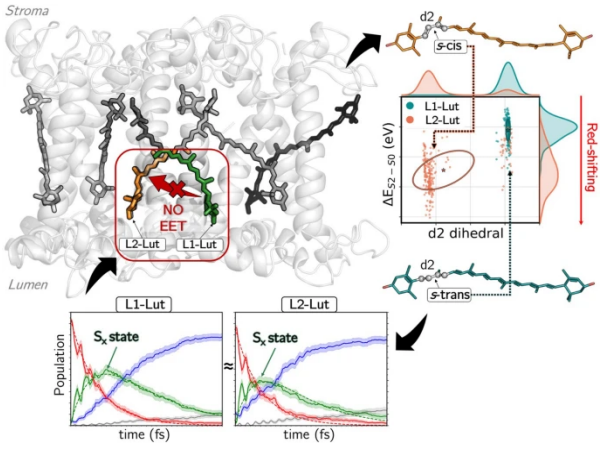

Figure 1: Graphical abstract [1].

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AMYC BIOMED 2024

New challenges in bioanalytical field

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Keywords: Liquid chromatography; ADC; forensic toxicology

Nowadays, one of the main objectives of analytical chemistry is to develop and validate methods in complex matrix, combining the principles of green chemistry with the newest fields of scientific interest. While efforts are being made to minimize energy, sample used and to use solvents and operator-safe procedures, the presence of complex bioanalytical matrices makes it more difficult. Conventional biological matrices such as blood, plasma and tissues useful in pharmacokinetic research, but also matrices that could be defined as unconventional represent practical examples of this. With the presentation we want to highlight that is possible to matches sensitivity and use of new matrices following a wide range of analytes, also with a common instrumentation, which doesn't need highly qualified staff [1].

With regard to the first area, somewhat known, the narrowest stumbling block is the use, increasingly frequent, of 'drugs' with great molecular weight in vogue in drug delivery. Interesting is, in fact, the possibility to follow an ADC (antibody-drug conjugate) in a complex system, such as the human body. In this research, we started from the simplest human matrix like plasma, choosing to follow the drug of interest using a reversed- phase column, a GraceSmart RP18 column. Additionally, cause of the nature of the drug, called DM4, which presents a thiol and through methyltransferases is directly methylated into S-Me-DM4. Thus, we developed the method for both, drug and its main metabolite, S-Me-DM4 [2]. The 'greenness' of the method is characterized by the only protein precipitation thanks to the addiction of Acetonitrile, following by drying of the supernatant, which is suspended and directly injected.

Forensic toxicology, on the other hand, is very different, and its interest in recent years has grown exponentially. In this research, the guanosine pathway was followed on an extremely new matrix, like extracellular vesicles (EVs) extracted from cadavers [3]. Their importance is due to the possibility of containing information about physio-pathological field. The choice of the matrix comes from the possibility of these EVs to help during the autopsy diagnosis. The complexity of this approach lies in the impossibility of having a 'blank' from which to start, but at the same time the interest has settled on the differences, in the quantification, of the target analytes extracting the EVs from different matrices, like urine, saliva and muscle. This method was born starting from an older one, already published [4], but during time we noticed that to follow the entire guanosine pathway was necessary to increase the analytes.

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Design of hypermodified NIR-responsive RNA conjugates for a spatio-temporal delivery of therapeutic oligonucleotides

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Keywords: organic chemistry, oligonucleotides, RNA, photolabile protective groups, photo-release

Significant advancements in precise cancer treatment are being driven by therapeutic oligonucleotides (TOs) through their ability to modulate cellular pathways, though effective delivery to cancer cells remains a major hurdle (1). Chemical modifications are crucial for overcoming biological barriers and enhancing TO delivery, which in turn improves their pharmacokinetic and pharmacodynamic properties (2).
In this research, we introduce an innovative method utilizing hypermodified NIR-responsive RNA conjugates for the spatio-temporal delivery of TOs. We have developed and optimized a synthetic strategy for a quinoline-based photo-caging compound using a convergent approach, obtaining in this way a two-photon excitable system using a quinoline moiety to encapsulate therapeutic RNAs (siRNAs and ASOs), facilitating controlled release upon activation. Additionally, by covalently linking the quinoline scaffold to a cysteine-selective molecular component, such as an antibody, we achieve tissue selectivity in our delivery system.
Our goal is to achieve precise spatiotemporal control over drug release by using a photolabile protecting group synthesized in our laboratory. Recent advancements in caging groups have optimized their absorption maxima for long-wavelength light, reducing phototoxicity, enhancing tissue penetration, and enabling multi-photon excitation for controlled drug release (3).
We also developed a library of five modified TOs (siRNA and ASOs) targeting the Smo receptor, which is crucial in the aberrant Hedgehog pathway expression seen in various malignancies, including Pediatric Medulloblastoma (4). This library will be integrated into our selective photo-labile system, enhancing its therapeutic potential.
Our pioneering approach combines chemical modification strategies with optochemical biology to advance targeted cancer therapy, offering promising avenues for clinical translation and improving patient outcomes.

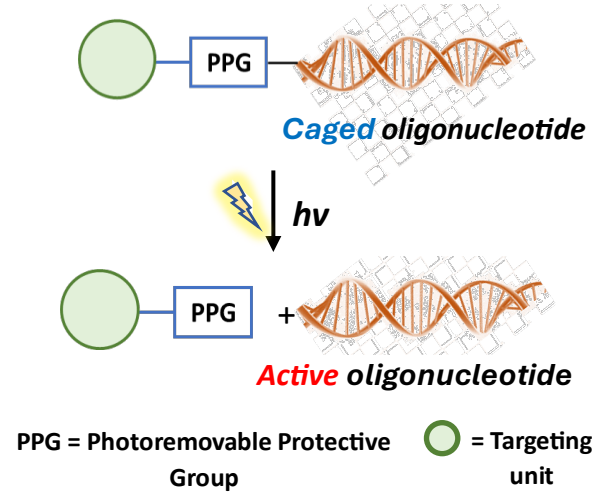

Figure 3 Schematic representation of the photo-release of a caged oligonucleotides in a system endowed with a targeting unit

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BIPHASIC POROUS STRUCTURES FORMED BY MONOMER/WATER INTERFACE STABILIZATION WITH COLLOIDAL NANOPARTICLES

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Keywords: Bicontinuous, drug delivery, colloids, codelivery

Bicontinuous jammed emulsion gels (known as bijels) are Pickering emulsion where the aqueous and organic phases are present as continuous phases [1]. These emulsions, stabilized by colloidal nanoparticles at the interface between the two phases, can be used in a variety of applications, especially drug delivery. The goal of this study is that of using a hydrophobic monomer, able to polymerize in bulk, thus forming a bicontinuous structure with polymer and water present as immiscible phases [2]. ε-caprolactone, selected as monomer, has been inserted in the reacting cylinder, along with TBD as catalyst. The system was mounted on an orbital shaker, and a stirring velocity of 1000 rpm has been set. Once the polymerization has occurred, an aqueous solution of NPs (both organic and inorganic have been tested) has been added, and the stirring speed has been increased up to 1700 rpm for 1 minute. Then, the stirring velocity has been decreased back to 1000 rpm until the bicontinuous structure formation occured. Release tests have been performed by soaking the bicontinuous structures in 2 mL of PBS at 37 °C for mimicking the physiological conditions. After certain timepoints, 1 mL has been withdrawn and replaced with 1 mL of fresh PBS. DOSY analyses were able to confirm the bicontinuity of such structures, and their mechanical and chemical properties have been fully characterized through different analyses (GPC, NMR, ESI-MS, DSC, Fluorescent confocal microscopy). Furthermore, the results obtained for release in PBS and solid media gave encouraging results. Important topic to be highlighted is the temperature control for the production protocol, since the final material strongly depends on it. These materials have been demonstrated able to load both hydrophilic and hydrophobic molecules and their release properties have been intensively studied. Through HR-MAS analysis diffusional studies have been performed, for tracking changes in release properties changing the NPs used (inorganic and organic). Furthermore, the possibility of codelivery of two different molecules (hydrophilic and hydrophobic respectively) has been characterized, highlighting an interaction between the two compounds that influence the final diffusivity values [4].

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Microfluidic Fabricated Liposomes for Nutlin-3a Ocular Delivery

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Keywords: Liposomes, Nutlin 3-a, microfluidic

Ocular drug delivery has always been a challenge research field due to the complex anatomic and physiological barriers present in the eye. These unique static and dynamic ocular barriers not only prevent the entry of foreign substances but also hinder the effective absorption of therapeutic agents. An ideal drug delivery system should enable improved drug bioavailability and controlled release at the target site, thus overcoming these ocular barriers. Novel drug-delivery technologies such as liposomes, are increasingly studied as potential ophthalmic drug delivery systems able to encapsulate and efficiently deliver also highly lipophilic drugs. In this regard, the present study aims to develop and produce liposomes (LIPO) formulation able to encapsulate and allow the ocular delivery of Nutlin-3a, a small non-genotoxic inhibitor of the MDM2/p53 interaction, that shows interesting therapeutical potential against retinal disease. LIPO were produced via microfluidic approach and their size distribution was evaluated by photon correlation spectroscopy and centrifugal field flow fractionation. Nutlin-3a entrapment capacity was evaluated via ultrafiltration and HPLC. Moreover, morphological, and structural characterization were conducted using transmission electron microscopy and Fourier-transform infrared spectroscopy, respectively. The microfluidic formulative study enabled the selection of LIPO constituted of phosphatidylcholine at concentrations of 5.4 and ethanol 10% ethanol, exhibiting a roundish vesicular structure with mean diameters around 150 nm, polydispersity index values always below 0.2, as well as high Nutlin-3a entrapment capacity [1].

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Online monitoring of urea in wastewater through biocatalytic electrochemical method

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Keywords: Urea, Biocatalysis, Wastewater, Design of Experiments, Response Surface Methodology.

In wastewater treatment facilities, enzymatic catalytic procedures can lessen contaminants and encourage environmentally friendly bioremediation techniques. Due to its extensive usage as a supplement to cow feed and fertilizer[1], urea levels in wastewater must be closely monitored. Soil runoff from these sources leads to environmental pollution, causing algal blooms and eutrophication[2]. In this work, we present a real-time urea measuring technique based on the combination of on-line potentiometric analysis and enzyme reactors. Flow-based bioreactors are the best choice for automated sample processing and ongoing monitoring. By immobilizing enzymes on solid-phase materials (such as glass beads or plastic tube inner walls)[3], a variety of bioreactors were built. These were then coupled to solid-state ammonium sensors for electrochemical measurements. Through the Design of Experiments approach, Response Surface Methodology was applied to evaluate the effect of glutaraldehyde concentration and urease amount and to find the best conditions for the assembly of a reactor while also minimizing material usage, all with a view to mass production. Analytical performance was assessed based on factors such as flow rate volume of injected sample and dilution factor. Key analytical parameters were evaluated, and the flow measurement was adjusted to produce the highest signal in the shortest amount of time. In the end, the on-line system ought to be entirely automated, making it possible to place it in key areas within wastewater treatment facilities for monitoring in real time.

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Hybridization chain reaction for enhancing miRNA detection at electrochemical strip

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Keywords: Hybridization chain reaction, miRNA, electrochemical biosensors, point of care

MicroRNAs (miRNAs) are small non-coding RNA molecules crucial for gene expression regulation and metabolic functions [1]. Due to their significant role in cellular processes, miRNAs are valuable as disease progression indicators and treatment targets [2,3]. Detecting and quantifying miRNAs is essential for understanding their biological roles and potential as biomarkers. Hybridization Chain Reaction (HCR) has emerged as a powerful tool for detecting and quantifying biomolecules, including microRNAs (miRNAs). HCR is an isothermal nucleic acid amplification technique that exploits the self-assembly of DNA hairpins to generate amplified signals in the presence of a target molecule [4,5]. In HCR, a trigger strand (the target molecule) initiates a chain reaction by opening hairpin structures, causing them to hybridize and form long DNA concatemers, significantly enhancing the detection signal and enabling identification at very low concentrations [6]. In this work, an electrochemical sensor using HCR was developed for the determination of miRNA-21, a biomarker associated with various diseases, including cancer, using a signal-off miRNA-21 detection platform. The unique aspect of this approach is that the electrode surface is not modified, simplifying the manufacturing process and reducing the potential variability associated with surface modifications. Instead, the sample is placed directly on the electrode surface, eliminating the need for complex surface functionalization procedures and reducing the overall cost and time required for analysis. Both types of DNA hairpins used in the sensor were modified with methylene blue (MB) as a redox mediator. In the absence of miRNA-21, the DNA hairpins remain closed, and the MB generates a measurable electrochemical signal. When miRNA-21 is present, it triggers the opening of the hairpins and initiates HCR, resulting in the formation of long DNA chains. These structures reduce the accessibility of the MB to the electrode, thus decreasing the electrochemical signal. The signal reduction is proportional to the concentration of miRNA-21, allowing detection at picomolar levels. The use of a polyester electrode provides a robust and cost-effective platform, demonstrating potential for point-of-care (PoC) diagnostics and real-time monitoring of miRNA-21 levels in clinical settings. The HCR technique was used to amplify the signal, thus enabling a highly sensitive detection. The platform was rigorously tested in both buffer and serum samples, demonstrating its versatility and robustness. The detection limit achieved was at the picomolar level, highlighting the exceptional sensitivity of the developed platform. This innovative platform exploits the principles of the hybridization chain reaction for the sensitive detection of miRNA-21, offering a simple, efficient and highly sensitive method with great potential for PoC diagnostics and real-time monitoring of miRNA levels in clinical settings.

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Application of synthetic approaches for discovering potential pan-hCoV NSP13 inhibitors

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Keywords: Pan-hCoV, SARS-CoV-2, helicase, NSp13, antiviral activity

Helicase of Human Coronaviruses (hCoVs) utilizes the energy of nucleotide triphosphate hydrolysis to catalyse the unwinding of double-stranded DNA or RNA in a 5′ to 3′ direction. Due to its highly conserved sequence and crucial role in viral replication, it is an attractive and promising target for drug development against various human coronaviruses (hCoVs), which may lead to mild self-limiting respiratory or serious infections and deadly diseases. [1] In this work, to identify new potential Pan-hCoV helicase inhibitors, the crystallographic structures of SARS-CoV-2 helicase available to date [2, 3] have been considered to rationally design a library of about 100 compounds that have been subsequently synthesized and characterized by structural (single-crystal X-ray diffraction) and spectroscopic (NMR, MS) methods. All compounds were found to inhibit both SARS-CoV-2 helicase-associated enzyme activities, namely NTPase and unwinding activity, showing IC50 values in the low micromolar range; among them, several compounds inhibited SARS-CoV-2 replication with low EC50 and no significant CC50 values. In addition, some of the most potent compounds exhibited pronounced antiviral activity against HCoV229E and MERS-CoV, highlighting some of them as promising Pan-hCoV helicase inhibitors.

These results suggest that the hCoV helicase is a valid target for developing new drugs to treat infection by SARS-CoV-2 and other HCoVs, potentially causing future emerging and re-emerging infectious coronavirus diseases.

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Diroximel fumarate loaded Solid Lipid Nanoparticles (DRF-SLN) as Potential Carriers for the Treatment of Multiple Sclerosis: Formulation Development and Optimization

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Keywords: fumarate, multiple sclerosis, lipid nanoparticles.

Fumarates have recently garnered considerable scientific interest for MS treatment because fumaric acid esters (FAEs) have shown effectiveness in treating autoimmune diseases through their immunomodulatory mechanisms. Dimethyl fumarate (DMF) and diroximel fumarate (DRF) are both orally administered prodrugs used in MS patients [1]. DRF, a second-generation drug, was developed to achieve higher efficacy with fewer side effects compared to DMF. Despite better GI tolerability, many patients still discontinue therapy due to the high occurrence of adverse events. As a result, there is a need for a suitable drug delivery system that can bypass the GI tract and avoid these problems. Direct nose-to-brain delivery has emerged as a promising strategy to circumvent the BBB and deliver drugs directly to the brain. However, the nasal mucosa's drug absorption is limited, and the nasal cavity's small volume makes nose-to-brain drug delivery challenging [2]. These issues can potentially be mitigated by using solid lipid nanoparticles (SLNs). SLNs offer effective nose-to-brain transport, improving drug bioavailability by increasing solubility, permeation, and stability, extending drug action, and reducing enzymatic degradation [3]. Furthermore, SLNs are considered nano-safe carriers as they are produced from physiological and biodegradable materials that are generally recognized as safe (GRAS). Therefore, the aim of research project was to develop an efficient formulation strategy to obtain stable and homogeneous SLNs loaded with DRF to be intranasally administered for the treatment of MS with the intention of improving brain targeting and patients' compliance.
DRF-SLNs were formulated by solvent-diffusion technique, using stearic acid as lipid phase and Lutrol F68® (Poloxamer 188) as surfactant. The choice to use this lipid matrix was carried out by in vitro MTT assay on neuronal cell line DPSC [4]. DLS data of DRF-SLN showed good technological parameters with a mean diameter of 210 nm, polydispersity index (PDI) value of 0.17 and zeta potential (ZP) value of -36 mV, predicting a good long-term stability. This particle size agreed with microscopy data. Moreover, the ability of SLN to act as carrier for DRF was evaluated and the mechanism to entry into the cells were evaluated, studying the interaction of SLN with biomembrane models (MLV).
Based on these promising results, the further objective will be to evaluate the effectiveness of the nanoformulation in vivo. Mice will be treated under late prophylaxis and therapeutic regimen and, at the end of the study mice will be sacrificed and brain, spinal cord, spleen and lymphonods will be collected to further histological and immunological studies.

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AMYC BIOMED 2024

Leveraging Cross-Linking Mass Spectrometry for Mapping Protein interactions at Plasma Membrane

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Keywords: XL-MS, embranomics, protein-protein interactions, regulatory networks, protein dynamics

Protein-protein interactions (PPIs) regulate every aspect of cell life, playing a pivotal role in terms of accurate operation of cell behavior through fine regulation mechanisms. Indeed, the consistent organization of PPIs networks increases exponentially the cell capability to control a plethora of molecular phenomena, including signal transduction, endocytosis, secretion and even cellular functioning or death. However, when this knotty balance between assembly and disassembly is hampered, the effects can impact on organs and tissue functionality [1].
It is a matter of fact that the identification of PPIs as potential pharmacological target in the treatment of several diseases represents a consolidate strategy in personalized medicine. Furthermore, advancements in biochemical field continuously unravel the PPIs complexities, providing deeper insights into cellular function and discovering potential therapeutic candidates. To dissect that molecular intricacy, cross-linking mass spectrometry (XL-MS) emerged as one of the most promising strategies to capture the dynamics of interactomes from small scale, such as macromolecular complexes to large scale, including vesicles, organelles and cells [2]. Since the plasma membrane proteins (PMPs) constitute ~60% of all drug targets [3], have access to the complete 'membranome' PPIs network would represent an unbiased approach to depict regulatory pathways controlling cell activities. Despite the biochemical impact, knowledge gaps regarding protein interactome at PM persist.
In this challenging context, we applied the XL-MS approach combined with PM purification pipeline to profile, on a system-wide scale, PM interactome of He-La cells in normal conditions. Specifically, we enriched PM- fractions from total cell lysates cross-linked before (native) and after (disrupted) PM dissolution. We defined the best experimental settings in terms of MS-cleavable cross-linker concentration, cross-linking reaction time, PM enrichment, MS acquisition and elaboration. Based on all detected inter-links, we assembled a protein global net composed of unique residue-to-residue pairs originating from more than 300 proteins assigned to the PM compartment. Then, we compared our experimental PMPs map to already annotated interactions and we revealed an overlap of 25%, confirming the accuracy of XL-MS dataset. With respect to PM-associated proteins, we targeted the highest number of PPIs for specific PM member families, counting ATP-binding cassette transporters, solute carrier transporters family, ATPase Na+/K+, Cu2+ and phospholipid transporters. Finally, we identified the remaining cross-links as part of proteins which are currently not described as interacting with each other, presenting potentially de novo partners. At present, we are implementing our XL-MS cell membranome workflow to unravel the existence of hidden disease-specific protein subnetworks in cell models of genetic metabolic disorders.

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The analysis of contaminants of emerging concern and trace elements in Louisiana red swamp crayfish suggests its possible exploitation trough sustainable applications

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Keywords: bioaccumulation, biomonitoring, environmental and biological matrices, freshwater, pollution

Abstract

Louisiana red swamp crayfish (*Procambarus clarkii*) is one of the most widely introduced freshwater species in the world, raising concerns about its impact on native aquatic biota [1]. Despite the important nutritional properties of freshwater decapods and their potential use as a source of bioactive molecules, there are potential health risks associated with their consumption due to their ability to accumulate contaminants [1].
In the present work, we aimed to analyze muscle and exoskeleton of *P. clarkii* and environmental matrices (water and sediment) for the presence of trace elements (TEs), pesticides, per- and polyfluoroalkyl substances, phthalates, antibiotics and cyanotoxins in three representative Sicilian aquatic ecosystems.
P. clarkii samples were divided into five pools to collect the necessary amounts of muscle and exoskeleton, and to increase the homogeneity and representativeness of measurements. After dissection of muscle (with and without intestine) and exoskeleton (with and without cephalothoracic region) samples were weighed, homogenized, and frozen at -20 °C until freeze-drying.
All extraction and analytical procedures were adapted to the type of matrix and analytes. Except for TEs, all samples showed concentrations below the detection limit for all contaminant classes. The bioaccumulation factor and the biotic sediment accumulation factor for TEs were, except in a few cases, below the EU bioaccumulative criterion. In addition, the PERMANOVA, PCA and HCA statistical analysis showed significant differences between the contamination profiles observed both between sites and between matrices, confirming that *P. clarkii* is a good bioindicator of TEs contamination. Nevertheless, the levels of TEs detected were comparable or lower than those reported in the literature [2] and below the maximum levels allowed by current European environmental and food safety legislation (EU Regulation 2023/915). However, these results are specific to these selected sites, time of sampling and contaminants and should be confirmed by further periodic sampling and analysis.
Based on these results, Sicilian *P. clarkii* individuals removed as part of monitoring, management or eradication activities required by EU and Italian legislation could be used as a resource in various sectors. Only in this exceptional case, the meat could be suitable for food or feed, while the exoskeleton, could be a valuable resource in various industries, such as the production of commercially valuable substances like chitin or chitosan. This would prevent the carcasses, which are considered waste, from having to be disposed of at additional environmental and economic costs, while also helping to cover the costs of managing this invasive species. In this context, the exploitation of *P. clarkii* could help to promote the policies and regulations necessary for more economically sustainable actions by local management authorities.

Acknowledgements

This research was financially supported by the project "Censimento, caratterizzazione, controllo e valorizzazione del Gambero rosso della Luisiana (Procambarus clarkii) in Sicilia (Mis. 2.49 art. 49 PO FEAMP SICILIA 2014 2020)", realised in cooperation between the dept. STEBICEF of the University of Palermo and the "Assessorato dell'agricoltura, dello sviluppo rurale e della pesca mediterranea" of Regione Sicilia. This research was supported by the fund “NextGenerationEU” of the European Union (D.M. 737/2021 CUP B79J21038330001 and by project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender

No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU - Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B73C22000790001, Project title "National Biodiversity Future Center - NBFC".

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Hyaluronic acid based-hybrid nanoparticles for targeted nose- to-brain delivery of dimethyl fumarate

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Dimethyl fumarate (DMF) (Tecfidera®) is a first-line oral therapy for relapsing-remitting multiple sclerosis, but patients often discontinue it within 3 months due to gastrointestinal discomfort [1]. Nose-to-brain drug delivery could be advantageous in increasing DMF brain availability and avoiding gastrointestinal exposure. Herein, hybrid nanoparticles loaded with DMF (H-NPs) were prepared using phosphatidylcholine, palmitoylethanolamide (PEA), cholesterol, and poloxamer and hyaluronic acid (HA). The H-NPs compositions were as follows: L and LD (without PEA and HA), LP and LPD (with PEA but without HA), LH and LHD (with HA but without PEA), and LPH and LPHD (with both PEA and HA) at different concentrations. PEA, an endogenous lipid mediator, primarily activates peroxisome proliferator-activated receptor (PPAR)-α, exerting anti-inflammatory, metabolic, and neuroprotective effects. HA, known for its exceptional mucoadhesive properties, selectively binds to RHAMM receptors, which are often overexpressed in many inflammatory conditions [2]. H-NPs were made by a modified nanoprecipitation technique. Size, zeta potential (ZP), morphology, total drug in dispersion, viscosity, mucadesions and thermal properties were studied. For 30 days, the chemical stability of DMF in the H-NPs and the physical stability of all H- NPs were explored. In-vitro DMF permeation studies were carried out by the PermeaPad® system. RPMI 2650 and SK-N-BE2 cell lines were used to investigate LP and LPH cellular uptake. The stability of DMF in human and rat blood was determined by setting appropriate extraction and HPLC procedures. The mean size of nanoparticles statisticallyincreased from 119 ± 1 nm of LP to 179 ± 4 of L at 219 ± 2 nm of LPH at 254 ± 2 nm of LH. Thus, HA and PEA influenced the physical properties of H-NPs. Also, the ZP has been most negative by HA and PEA addition: from -2,3 ± 1 mV of Lto -17,3 ± 1 mV of LP to -25 ± 1 mV of LPH at -29.9 ± 1 mV of LH; TEM images proved that H-NPs were spherical but with irregular surfaces. Thermal analysis revealed the presence of HA on the H-NPs surface. The formulations showed great chemical-physical stability for 30 days. The total amount of DMF in dispersion was about 60%. H-NPs showed viscosity suitable for nasal administration regardless of HA presence. The permeation studies indicated that LHD and LPHD increased the amount of DMF permeated more than LPD and LD and free-DMF, evidencing the influence of HA on H-NPs performance. LP and LPH showed a rapid cell uptake at three hours. Compared to human blood, DMF appears immediately and totally degraded in rat blood, where it is partially hydrolysed to MMF. These results point to the potential use of H-NPs as viable nanocarrier candidates for encapsulating DMF for intranasal delivery and support the work in progress aimed at in vivo pharmacokinetic assessment. and support the work in progress aimed at in vivo pharmacokinetic assessment.

Keywords: Dimethyl fumarate ;Hyaluronic Acid; polymeric-lipid nanoparticles; intranasal administration

Acknowledgements: Support by Bando Fondazione di Sardegna – 2018-2020 e 2021 – Progetti di ricerca di basedipartimentali; Grant numbers J89J21015120005.

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Development of innovative MIP based sensors for liquid biopsy

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Keywords: Biosensors; Molecularly imprinted polymers; liquid biopsy.

The development of suitable, simple and practical diagnosis methods for cancer biomarkers detection is largely related to huge demands that are emerging in medical diagnostics. In this sense, biochemical sensors yielded significant advancement in the field of biomedical analysis. In the biosensor design, molecular recognition is a crucial part and a fundamental property of biological processes and has been demonstrated to be a powerful analytical tool in the form of antibody/antigen recognition and enzymatic catalysis. However, systems based on natural recognition elements have several drawbacks, such as high cost and low stability. Thus, the development of biomimetic receptors able to replace natural antibodies and which can offer improved stability, cost-effectiveness and means of rapid fabrication has received a great deal of attention due to the performing capability of analyte-recognition [1]. In this respect, molecular imprinted polymers (MIPs) are synthetic receptors utilized as memetic antibody for selective molecular recognition which offer valuable opportunities for biosensing purposes providing templates able to non-covalently bind to antigens with the corresponding (imprinted) molecular morphology. This work is focused on the integration of MIPs into miniaturized devices, for the development of easy and portable devices for liquid biopsy with the aim to increase the life expectancy of patients.

In a first work, the artificial macromolecular receptor for TGF-β1 based on molecular imprinting was synthesized by electro-polymerization of the functional monomer, o-phenylenediamine (o-PD), in the presence of TGF-β1 as the target molecule on single use interdigitated Platinum microelectrodes. The MIP based sensor demonstrated high selectivity and high sensitivity with a linear response to the TGF-β1 concentration in buffer solution and a LOD of 0.09 ng ml^{-1} (Figure 1 A) [2].

The same procedure for MIP synthesis was exploited for the development of an optical biosensor utilizing porous silicon thin layer, properly tailored for the specific recognition of Interleukine 6 (IL-6). The incorporation of MIP on the porous silicon sensor surface enhances its capability to selectively capture and detect IL-6, making it a promising tool in the field of diagnostics. The electrodeposition of MIP onto the silicon porous structure ensures a tailored and specific binding interface for IL-6, contributing to the sensor's heightened sensitivity and selectivity (Figure 1 B).

Finally, we developed a BIC sensor modified with a cladding layer of polydopamine (PDA), meticulously crafted to recognize and bind specifically to TGF-β1, and serving as the molecular recognition element of the sensor. The local field enhancement and confined light within the BIC intensify the interaction with the imprinted sites on the polymer, amplifying the signal associated with TGF-β binding. The TGF-β1 detection in a complex matrix of spiked saliva with a LOD = 10 fM, and a very high resolution of 0.5 pM at physiological concentration using the optical lever readout (Figure 1 C).

The novelty of our MIP-based approach relies on the fact that the developed sensors are sensitive and selective: in fact, they can detect molecules at very low concentrations and demonstrated no unspecific interaction with interfering molecules and complex biological matrices.

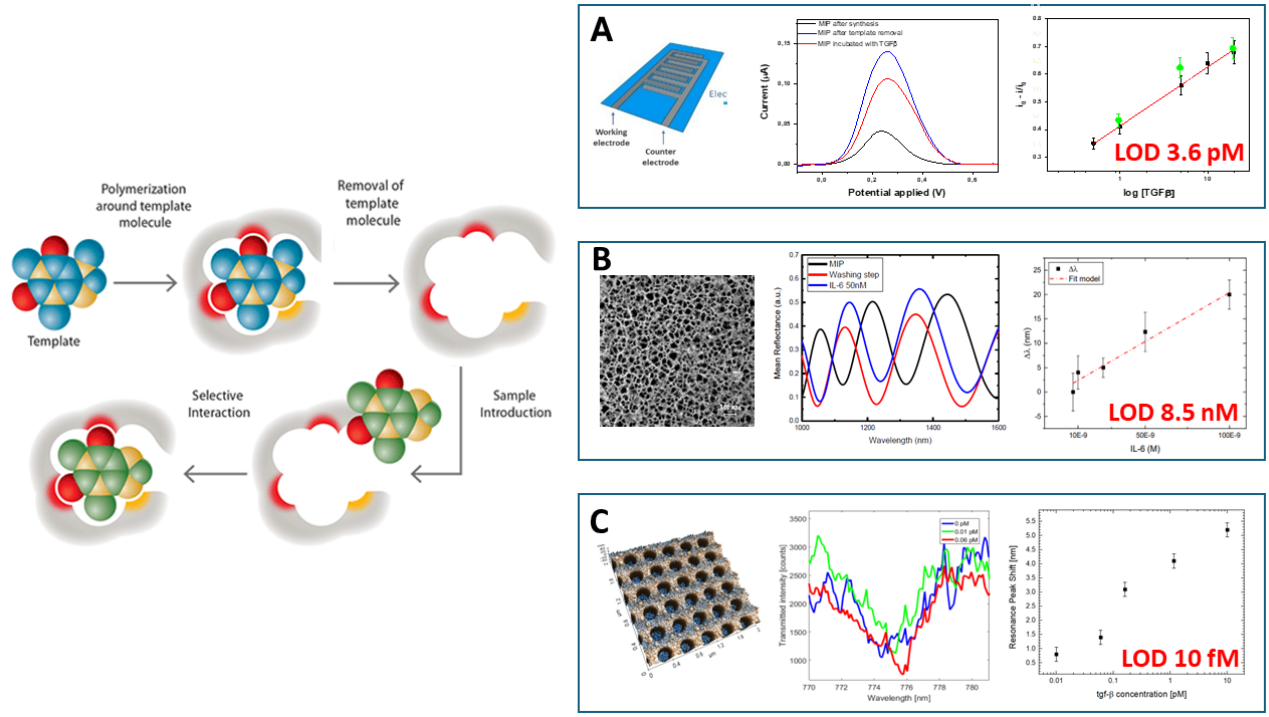

Figure 1. Schematic representation of MIP synthesis mechanism; A) DPV graphs of the MIP synthetized on interdigitated Pt electrodes after electro-polymerization (black line), after template removal (blue line) and after incubation with TGF-β1 20 ng mL^{-1} (red line) and calibration line in PBS buffer (red line) and in artificial saliva (green spots); B) Reflectance spectra of MIP synthetized on porous silicon (black line), after template removal (red line) and after incubation with IL-6 50 nM (blue line) and calibration line in PBS buffer; C) Transmittance spectra of MIP synthetized on BIC sensor (blue line) and after incubation with different concentration of TGF-b1 (0.01 pM green line, 0.06 pM red line) and calibration line in PBS buffer.

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Stencil printed sensors on paper from algae wastes for bisphenol A determination in toxicological studies conducted on zebrafish embryo model

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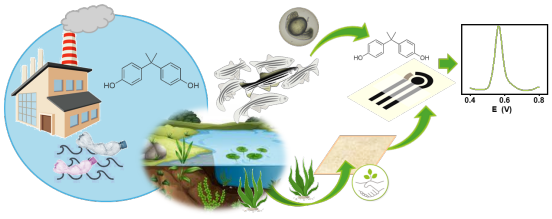
Keywords: electrochemical; biochar; in-vivo studies; contaminant analysis;

Bisphenol A (BPA) has become a prevalent contaminant in environmental waters given its massive industrial use, posing direct and indirect risks to aquatic ecosystems and human health. In this work, paper deriving from seaweed biomasses wastes (Algae paper) has been harnessed for the manufacture of lab-made electrochemical sensors via stencil-printing; the sensors were then employed to assess the BPA bioaccumulation in zebrafish embryos (Z-EBs; *Danio rerio*) and in their culture medium.

Firstly, the capability of Algae paper to assist the stencil printing was proved, testing different graphitic inks as conductive elements; the stencil printing was attempted also on other 'recycled' and office papers, and polyethylene terephthalate (PET) was used as control. Algae paper results the more prone to assist this printing technique, returning reproducible and superior performance. Besides, to enhance the electrochemical features of the sensors, two types of biochar (BH), deriving from industrial by-products, with amorphous (A) and nanofibrillar (F) morphologies were employed; both BHs were dispersed employing a solvent-free sonochemical method (liquid phase exfoliation) in aqueous phase.

The most performing sensor was selected and used as a monitoring tool during ecotoxicological studies conducted exposing Z-EBs to increasing levels of BPA. The sensor proved the ability to determine BPA in Z-EBs culture medium, returning useful sensitivity (LOD = 48 nM) and response linearity (0.25 – 6 µM), along with remarkable accuracy (recoveries ranged between 95-113%). Eventually, the Algae paper sensor was challenged for the determination of the BPA bioaccumulated in Z-EBs. To this aim, calibrations and recoveries studies were conducted in uncontaminated Z-EBs (blank matrix), satisfactory sensitivity (LOD = 57 nM) and signal linearity (0.25 – 8 µM) were obtained, together with quantitative recoveries (94-118%). Afterwards, the Algae paper sensor was used to detect BPA accumulated in Z-EBs exposed for 96h to different concetration of BPA (from 0.5 to 8 mg L^{-1}). The algae sensor was able to effectively distinguish between non-toxic and subtoxic levels, and when high concentrations of BPA were detected, Z-EBs showed morphological damages, tares, and growth delays.

Herein, the combination of Algae paper and BH allowed the production of an effective tool for monitoring BPA levels in Z-EBs model, allowing the enviromental tracking of the contaminant and the discrimination of toxic effects.



Acknowledgements: This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY

Portable Electrochemical Sensor for Hydrogen Peroxide Detection for Assessing Oxidative Stress in Disease Models

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Keywords: Oxidative stress; reactive oxygen species; electrochemical sensor; nanomaterials

Abstract

Reactive oxygen species (ROS) are molecules, with oxygen, that can react chemically. These species can exhibit reactivity due to the presence of unpaired electrons, which is typical of radical species. However, ROS also include reactive non-radical species, such as hydrogen peroxide (H_2O_2). The activity of ROS can result in stress, subsequently causes damage to DNA and other macromolecules. This damage is associated in the beginning and advancement of numerous diseases, such, cardiovascular diseases, neurodegenerative conditions, and cancer, as well as being a factor in the normal aging process. Given the significant impact of oxidative stress on health, there is a pressing need to develop a sensitive analytical technique for detecting ROS in biological sample. H_2O_2, a ROS is particularly of interest for researcher because of its involvement in causing oxidative stress. In response to this need, a portable point-of-care (PoC) electrochemical system based on on reduced graphene oxide-cobalt oxide-platinum (rGo-Co_3O_4-Pt) nanocomposites has been developed to detect H_2O_2 in cell lines induced with oxidative stress in cardiac tissue by using doxorubicin. This sensor is designed to be affordable, disposable, and highly selective for H_2O_2 detection, making it a practical tool for clinical and research applications. The sensor demonstrates a dynamic linear range of up to 2.5 µM with a limit of detection (LOD) of 0.8 µM and a limit of quantification (LOQ) of 2.5 µM. The sensor was evaluated for its practical application by measuring ROS levels in cell lines affected by doxorubicin induced cardiotoxicity and, normal cell lines. The comparative highlights the capability of the newly developed sensor to differentiate between varying levels of oxidative stress in different cell environments. In conclusion, the findings suggest that this portable electrochemical system can provide a reliable and efficient method for ROS detection, offering significant implications for managing diseases associated with oxidative stress.

Development and application of green sample preparation for non-targeted semi-quantitative metabolomics analysis of Melissa Officinalis leaves.

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Keywords: green extraction, NADES, natural products, metabolomic workflow, open-source software

This study investigates the potential of green techniques for extracting bioactive compounds from Melissa officinalis (MO) leaves. Specifically, we focus on the synthesis and application of twenty-one Natural Deep Eutectic Solvents (NADES) with a relative polarity ranging from 0.34 to 1.29.

Their extraction affinity against a set of 13 plant metabolites was predicted using COSMO-RS software [1] and experimentally validated using quantitative LC-HRMS metabolomics analysis. Subsequently, a non-target analysis was performed to explore the chemical diversity and complexity of the obtained extracts. Data processing was performed using open-source software tools such as MZmine 3.9.0 for data preprocessing and feature alignment [2] and SIRIUS+CSI:FingerID for metabolites annotation [3]. Overall, 249 and 195 metabolites were annotated in positive and negative ionization ion mode, respectively. Additionally, MS2Quant, a machine learning model that enables prediction of concentration from fragmentation (MS^2) spectra of detected chemicals, was used to perform semi-quantification of 201 $[M+H]^+$ annotated analytes [4]. The results underscore the efficacy of employing various types of NADES with different polarity ranges for selectively extraction of biochemical classes, providing valuable insights into the composition and concentration of bioactive compounds. In conclusion, this study demonstrates the feasibility and potential of utilizing green extraction techniques, particularly NADES, for extracting bioactive compounds from plants. The comprehensive mass spectrometry analysis elucidates the chemical composition and bioactivity of the obtained extracts, paving the way for further exploration and utilization of green extraction methodologies in natural product research and small molecules discovery.

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Investigating Cell Receptor Targeting with Functional DNAOrigami in Bio-Nano Systems

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Keywords: DNA Origami, Cell receptors, Aptamer, Cell imaging, Bio-nano interactions

The DNA origami technique facilitates the efficient assembly of customizable DNA structures, allowing precise arrangement of various biomolecules at the nanoscale. Due to their inherent compatibility with biological systems and the capacity to control shape, size, and arrangement of ligands, DNA-based nanostructures hold significant potential for applications in nanomedicine [1,2]. Despite successful demonstrations of binding in diverse scenarios, the underlying biophysical dynamics governing membrane interactions remain largely unexplored.

In this investigation, we delve into the binding kinetics of two rod-shaped DNA origami nanoconjugates: one decorated with anti-EGFR (Epidermal Growth Factor Receptor) antibodies and the other with anti-EGFR aptamers. Utilizing single-molecule tracking (SMT) microscopy, we track the trajectories of ligand-modified DNA origami bothin free solution and when bound to the cell membrane. This allows us to calculate their diffusion coefficient (D) anddiscern the fraction of DNA origami specifically bound to the targeted receptor, distinguishing them from nonspecific bindings. Moreover, SMT enables determination of the first-order rate constant for complex dissociation (Koff), a crucial parameter indicating the average duration of interaction between the DNA origami and the membrane receptors. Fine-tuning Koff values holds importance for modulating potential therapeutic and stimulation effects [3]. Our findings reveal that our DNA origami nanoconjugates exhibit specific binding with a Koffprofile potentially enabling preferential binding to cancer cells, thus presenting new avenues for DNA origami-based selective cell targeting in biomedical applications.

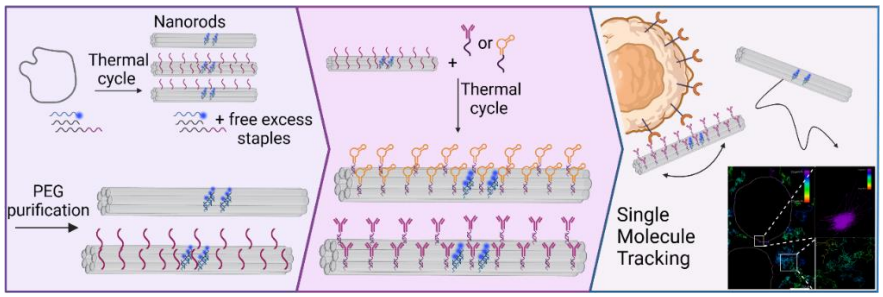

Fig.1: Schematic rendering of the workflow from DNA origami preparation to in vitro microscopy studies.

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Lycopene extraction with α-pinene, Natural Volatile Deep Eutectic Solvent Menthol-Thymol and Extra Virgin Olive Oil

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Keywords: *lycopene, extraction, EVO oil*

A completely green, easy and no time consuming method to extract lipophilic compounds from food waste was developed using terpenes as green solvents and ultrasound-assisted extraction as green technique [1]. The main finding of this research was the possibility to evaporate the DES solvent with the recovery of the dried extract similarly to the use of other solvents as *n*-hexane and α-pinene. The weakness of this method is the loss of lycopene during the evaporation. Despite this partial degradation, the quantity of lycopene in the dried extract is high. The application of this deep eutectic solvent is relatively unexplored, thus it can be used for the extraction of hydrophobic molecules less sensible to heat. Simultaneously, we used the ultrasound-assisted extraction with extra virgin olive oil as extraction solvent which allows the production of an enriched oil with improved antioxidant and radical scavenging activities (Figure 1). We enriched an EVO oil with the aim to delineate a possible future nutraceutical application, opening new perspective in the use of food waste as source of beneficial compounds. For example, this enriched oil could be used as food seasoning. The ideal daily dose of lycopene is not defined yet, but according to different epidemiological studies, daily lycopene intake should be from 2 to 20 mg per day. Following the EFSA guidelines [2], about 10g of enriched EVO oil should provide 9 mg of lycopene, a quantity that perfectly fits the range established by EFSA.

Figure 1. Lycopene enriched EVOO.

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Boolean Logic Network based Biosensor for Wine Adulteration Detection

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Keywords: Boolean Logic Gate biosensors, enzymatic biosensors

In the last decades, much progress in the bioelectrochemistry of redox enzymes has been achieved through the synthesis/electrosynthesis of new nanomaterials to enhance the efficiency of direct electron transfer (DET) between electrodes and several sugar oxidising enzymes (e.g., cellobiose dehydrogenase (CDH), pyranose dehydrogenase (PDH), fructose dehydrogenase (FDH), PQQ-dependent glucose dehydrogenase (PQQ-GDH) etc.).[1,2] Besides the evolution of the electrode construction, one of the most intriguing achievements was reported in 2001 by Willner and Katz, who coined the new definition of "self-powered biosensors" correlating the power generation with different glucose concentrations.[3]

In this work, we developed a self-powered biosensor for the detection of sucrose by combining an anodic electrode modified with fructose dehydrogenase (FDH), PQQ-dependent glucose dehydrogenase (PQQ-GDH) and invertase/mutarotase to create a catalytic cascade that is logically operated to oxidise sucrose. After the machine optimization, all the enzymes were physically immobilized onto the multi-walled carbon nanotubes (MWCNTs) modified electrode surface by using the photo cross-linking of poly(vinyl alcohol), N-methyl-4(4'-formylstyryl)pyridinium methosulfate acetal (PVA-SbQ). In addition, the logic operation of the anode related to a cathode electrode modified with glucose oxidase and hemin.[4] The proposed self-powered biosensor was able to detect sucrose with a sensitivity of 48.2 ± 0.2 μW cm^{-2} mM^{-1} with a dynamic linear range up to 500 μM. The so prepared self-powered biosensor was used to detect wine fraud through the addition of sucrose to enhance the content of reducing sugar (adulteration of wine samples).[5]

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Carbon Dots-poly(ethylene glycol)-folate conjugates for the targeted and NIR-triggered release of doxorubicin in the treatment of breast cancer

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Keywords: carbon dots, nanotheranostics, targeted delivery, hyperthermia, breast cancer.

Breast cancer (BC) remains one of the most prevalent cancers globally which poses a persistent challenge in modern healthcare, prompting a shift towards precision approaches based on a deeper understanding of the disease. These strategies enable real-time monitoring (imaging) and patient-specific solutions (therapy) [1]. In this context, carbon dots (CDs) are emerging due to their versatility, biocompatibility, and intriguing optical properties, including high fluorescence and near-infrared (NIR) photothermal conversion, which can induce cell damage [2]. A promising strategy could thus be the CDs-based theranostics (therapy+diagnosis), which offers potential intervention at various stages of BC progression, aiming to tackle chemoresistance by using a single device for both drug delivery and photothermal therapies, coupled with real-time diagnostic monitoring of the outcomes, to achieve better spatiotemporal control over therapeutic activity [3].

Therefore, the aim of this work was to develop a theranostic nanosystem for the active targeting of the antineoplastic drug doxorubicin, with potential application in Imaging-Guided Photothermal Therapy (IG-PTT) for BC treatment (Fig.1). The designed nanosystem consists of carbon dots (CDs) obtained through solvothermal synthesis from urea, citric acid and indocyanine green [4], surface-passivated with 1,2-Bis(3-aminopropylamino)ethane (bAPAE), and finally PEGylated with polyethylene glycol chains bearing folic acid (FA) residues, resulting in the system named CDs-bAPAE-PEG-FA. The drug-free nanosystems showed a functional polymeric shell that yielded to a hydrophilic nanocarrier with mean diameter of about 12 nm and a high surface area available for drug interactions. Fluorescence analyses demonstrated that the passivated nanosystems could be used as fluorescent probes in bioimaging, retaining the multicolor fluorescence of the starting CDs. Moreover, CDs-bAPAE-PEG-FA presented good photothermal conversion after NIR exposure, suggesting their potential use in the PTT of BC. The subsequent incorporation of doxorubicin enabled the production of the final theranostic nanosystem (CDs-bAPAE-PEG-FA/Dox0). The drug-loaded CDs exhibited NIR-boosted release, increasing the amount of drug released by 50% compared to samples not treated with NIR light.

Furthermore, in vitro studies on the MCF-7 and MDA-MB-231 BC lines highlighted the fundamental role of NIR stimulation, which substantially enhanced antitumor efficacy, indicating a possible use of this nanoplatform for on-demand drug release right at the tumor site. Finally, uptake studies demonstrated the efficient internalization as well as the crucial function of the targeting agent FA, showing a decrease in system internalization after saturation of the folate receptor. Therefore, the use of CDs-bAPAE-PEG-FA/Dox appears promising for potential application in IG-PTT and selective drug delivery of BC, enhancing the efficacy of the antineoplastic treatment.

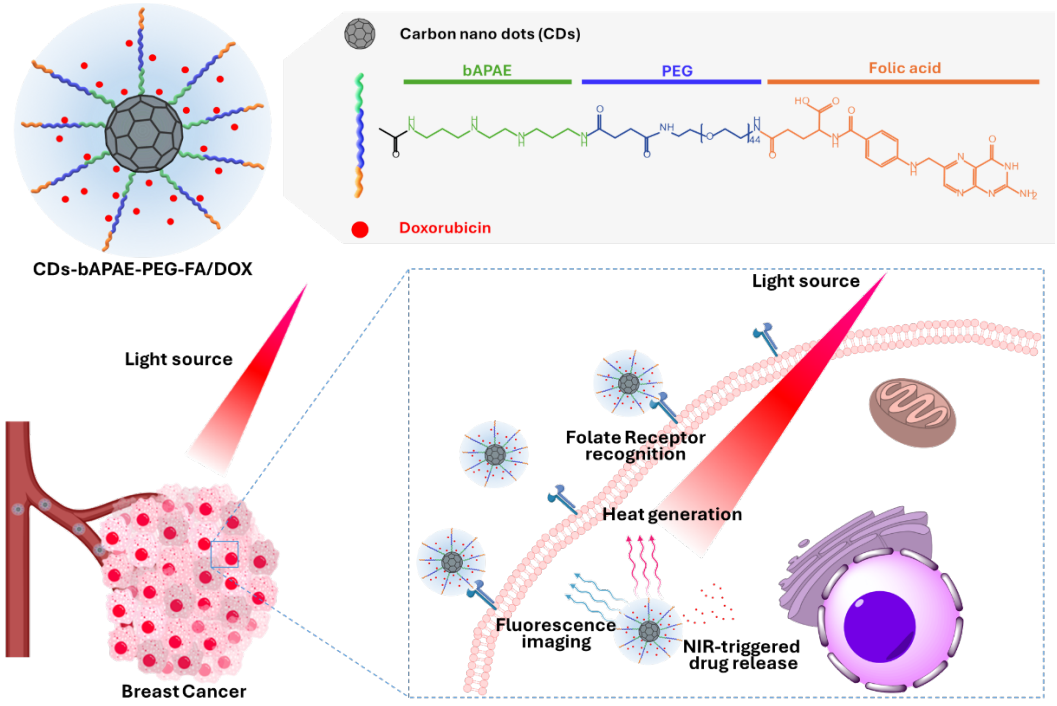

Figure 1. Schematic representation of the aim of the work.

Acknowledgements

Paola Varvarà was supported by Fondazione Veronesi.

Paola Varvarà thanks PNRR PE6 "HEAL ITALIA" (PE0000019-CUP UNIPA B73C22001250006) for the financial support.

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Multimethodological Approach for the Assessment of Apple Samples in Commercial Bio-Packaging

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Keywords: apple; biofilm; metabolomics.

A comprehensive quality control system of food is required to monitor each step of the chain, from production to retail, ensure environmental and human safety and improve consumer's health. Chemical and metabolic transformations deriving from the effects of (new) manipulations, storage and packaging conditions may largely affect the composition of traditional and novel foods. Otherwise, food packaging plays a crucial role in maintaining food quality during its distribution. The conventional use of plastic materials in packaging contributes significantly to environmental pollution and may pose a food contamination risk. Therefore, there is a pressing need to adopt environmentally friendly biofilm materials in food packaging to address these concerns. [1]. In this scenario, the present study was carried out in the frame of the ONFOODS consortium [2] (Research and Innovation Network on food and Nutrition Sustainability, Safety and Security – Working ON Foods) stemming from the National Recovery and Resilience Plan (NRRP), and is aimed at the metabolite profiling and monitoring the preservation of conventional apples (Melinda Golden Delicious) in commercial biofilms, for the improvement of apple quality that emerges as a matter of priority for customer's safety. Apple slices have been stored in three different commercially available packagings: **A** from corn starch, cassava and eucalyptus, **B** made of polylactic acid from corn starch, and **C** a polyethylene film used as reference [1]. Analyses to monitor chemical profile and new and (re)-emerging hazards in apple sample at three time points (0, 14 and 21 days) were carried out by means of untargeted (FT-ICR MS, NMR) and targeted methodologies (HPLC-DAD, HPLC-MS/MS). In particular, the high sensitivity and mass accuracy typically achieved with FT-ICR MS implies that elemental formulas of many metabolites and harmful compounds present in trace amounts, like pesticides, agrochemical derivatives and metals, can be determined. Overall, a broad chemical and metabolomic profile have been gathered to monitor traceability and quality of apples.

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Engineering intravitreal implant combining microfluidics and 3D-printing techniques for synergistic delivery of Sirt1 agonists

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Keywords: Liposomes, hydrogel, hyaluronic acid, diabetic retinopathy, sustained release

Treating diabetic retinopathy (DR) is challenging due to poor drug absorption after topical applications and limited drug diffusion through the ocular barriers, necessitating high doses with systemic side effects. Intraocular injections remain the gold standard treatments, due to their direct delivery, but it is associate with several drawbacks, including invasiveness and patient discomfort [1]. Therefore, polymeric implants are being researched to provide long-term therapeutic drug levels for posterior eye disorders with high efficacy and minimal discomfort [2, 3]. The current study aims to develop a biocompatible, soft, minimally invasive, biodegradable, and bioadhesive material-based hydrogel scaffold to prevent common issues with implants [4]. A grid-shaped scaffold was developed using coaxial 3D printing (3DP) to extrude two different hydrogel bioinks in a single filament. Two molecules at a time are delivered within the systems. Curcumin (CUR) and Resveratrol (RSV) are chosen as model molecules for their synergistic activity as agonists of Sirt1, a protein downregulated in DR. The scaffold comprises an inner core of curcumin-loaded liposomes (CUR-LPs) that are prepared by microfluidics (MFs) embedded in a hydrogel of hydroxyethyl cellulose (HEC), and an outer layer of hyaluronic acid-chitosan matrix with free RSV. Optimized liposomes, prepared via MFs, exhibit suitable properties for retinal delivery in terms of size (<200 nm), polydispersity index (PDI) (<0.3), neutral zeta potential (ZP), encapsulation efficiency (~97%), and stability to up to 4 weeks. Mechanical studies confirm scaffold elasticity for easy implantation. Different release patterns are observed due to different localization of the molecules inside the scaffold resulting in burst release for RSV and slow release for CUR. Microscopic analysis confirms optimal distribution of liposomes within the hydrogel scaffold and cytocompatibility with human retinal endothelial microvascular cells (HREMCs). Liposomes and hydrogels can generate dual drug-loaded 3D structures that shows promise for sustained ocular drug delivery, reducing the need for frequent intraocular injections and enhancing patient comfort and treatment adherence.

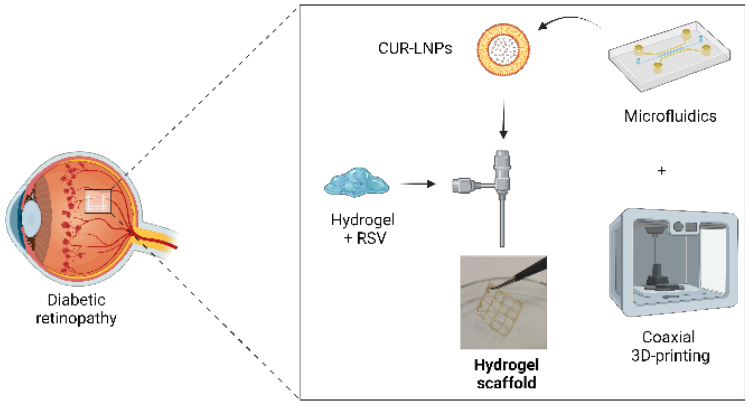

Figure 4. Schematic production of hydrogel scaffold combining microfluidics and 3D printing.

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SLAM

A magnetic bead-antibody bait for selecting aptamers against*Mycobacterium tuberculosis* secretory antigen ESAT-6/CFP-10 heterodimer for tuberculosis rapid test development

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Keywords: aptamers, SELEX, tuberculosis, ESAT-6/CFP-10, biorecognition elements

A significant majority of individuals infected with tuberculosis (TB) remain undiagnosed each year. TB diagnosis still heavily depends on the analysis of sputum samples, yet not all TB-infected individuals can expectorate sputum[1]. While there has been progress towards rapid TB diagnosis, biomarker-based rapid tests using non-sputum samples remain a pressing need for TB detection. The possibility of detecting *Mycobacterium tuberculosis* secretedantigens such as ESAT-6, which is in complexed with CFP-10 necessary for the bacterium's virulence, holds great diagnostic potential as this can be identified as a predictor of TB in easily accessible samples, such as urine and blood [2]. However, an efficient approach in choosing a biorecognition element to identify these antigens is necessary for accurate and specific rapid test development.

Here, we present the generation of aptamers, nucleic acid-based biorecognition molecules, against the ESAT-6/CFP-10 heterodimeric complex aiming at the discovery of aptamers that could be subsequently used in sandwich-based rapid tests. The two TB recombinant antigens were prepared individually, the complex was formed *in vitro* and it was characterized by native PAGE and intrinsic tryptophan fluorescence spectroscopy prior to the selection. By employing a modification on the generic aptamer selection process called systematic evolution of ligands by exponential enrichment (SELEX), the target-bound single stranded DNA (ssDNA) sequences were baited with an α-CFP-10 IgG antibody immobilized on magnetic beads for isolation of sequences forming a sandwich with the immobilized antibody. We termed this approach as "Pulldown-capture SELEX". To achieve this, three-pronged approaches were performed – selection, identification, and characterization of heterodimer aptamer binders. First, during the selection, the ssDNA library was immobilized onto magnetic beads
via hybridization with a partially complementary docking probe. When the ESAT-6/CFP-10 target complex is added,ssDNA is displaced from the beads to the solution to facilitate binding to the target and are magnetically separated from the beads and the unbound sequences. These eluted target-bound ssDNA sequences are then mixed with magnetic beads with an immobilized α-CFP-10 IgG antibody, thereby isolating the sequences that have high affinity for ESAT-6 and form a sandwich with the antibody. Second, the aptamer sequences were identified bynext generation sequencing to identify enriched aptamer sequences. Lastly, the binding properties of the identified aptamers were characterized by enzyme-linked aptamer assay to validate their affinity for the ESAT-6/CFP-10 heterodimer. . Taken together, by introducing the antibody-magnetic bead pulldown, the selection process and specificity and affinity of the candidate aptamers are enhanced, thus producing biorecognition

elements with high selectivity towards their cognate targets. The strategies presented offer a new approach forselecting aptamers for use in a sandwich assay moving towards non-sputum-based detection of tuberculosis.

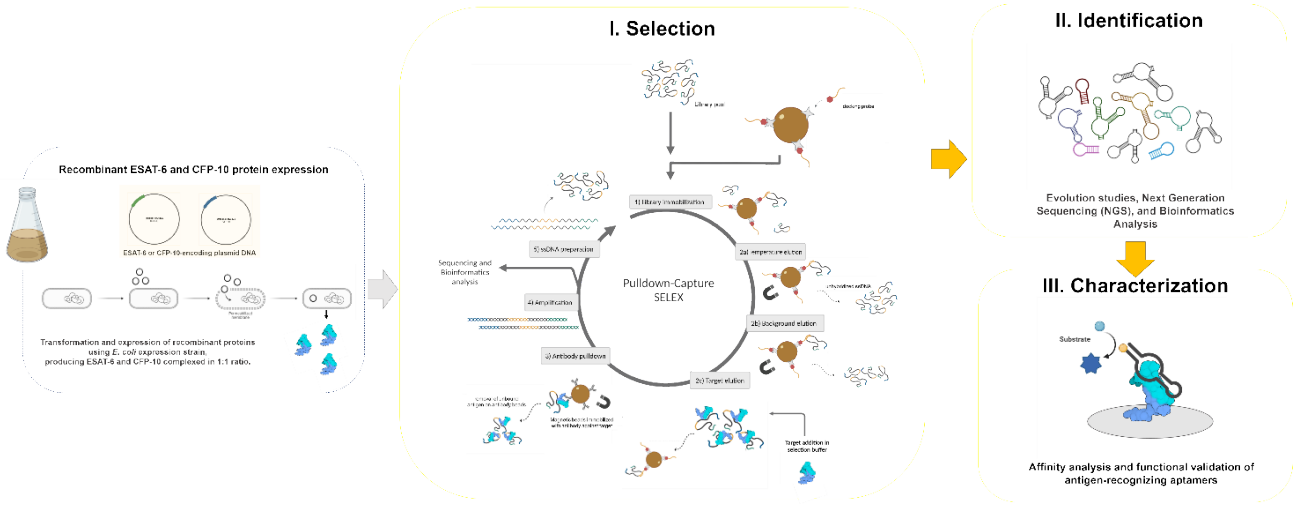

Figure 1. Pulldown Capture SELEX approach for the selection of aptamers against *M. tuberculosis* ESAT-6/CFP-10 heterodimer protein.

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SUSTAINABLE JOULLIE'-UGI AND CONTINUOUS FLOW IMPLEMENTATION LED TO NOVEL CAPTOPRIL-INSPIRED BROAD-SPECTRUM METALLO BETA-LACTAMASE INHIBITORS

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Keywords: Metallo beta-lactamases, antibiotic resistance, spiroindoline, flow chemistry.

Antimicrobial resistance is one of the top global public health and development threats. It is estimated that bacterial AMR was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths. The misuse and overuse of antimicrobials in humans, animals and plants are the main drivers in the development of drug-resistant pathogens. The β-lactam antibiotics including penicillins, cephalosporins and carbapenems are the cornerstones of antimicrobial chemotherapy.[1] ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*) have become highly resistant to most available antibacterial drugs by exploiting different mechanism of resistance.[2] The COVID-19 pandemic has increased the antibiotic resistance, since COVID-19 patients were treated with broad-spectrum antibiotics, including extended-spectrum cephalosporins, quinolones, and carbapenems.[3] Several mechanisms of resistance can be identified including i) inactivation of the drugs by enzymes, ii) increased expression of efflux pumps, iii) modifications in the cell wall, and iv) target modifications. One of the main mechanisms of resistance is the expression of metallo beta-lactamases (MBL), that are bacterial enzymes able to cleave the beta-lactam ring of the antibiotics. Since no clinically relevant MBL inhibitors have yet been approved, the quest for novel compounds with a broad-spectrum activity against several MBL, represents a necessary although challenging task. Captopril, an angiotensin converting enzyme inhibitor used for the treatment of hypertension, showed weak inhibitory activity against some MBL isoforms. Therefore, our driving idea was to rationally modify the structure of the captopril to improve potency and possibly extend the spectrum of inhibition towards MBL by increasing the hydrophobic features of the molecule generating an indoline system. We employed a Joullié-Ugi multicomponent reaction protocol for the synthesis of two indoline-based subseries, generating a novel class of potent MBL inhibitors through a convenient continuous flow protocol for the rapid, efficient and diversity-oriented generation of analogues. Among the compounds tested against different MBL isoforms, two derivatives (compounds **1a** and **1b**, Figure 1) stood out for their broad-spectrum inhibition profile against three MBL isoforms, namely NDM-1, VIM-1, and IMP-7. Furthermore, activity in clinical isolates in synergy with beta-lactam antibiotics was assessed for the best performing compounds, thus paving the way to further optimization for the newly disclosed inhibitors.

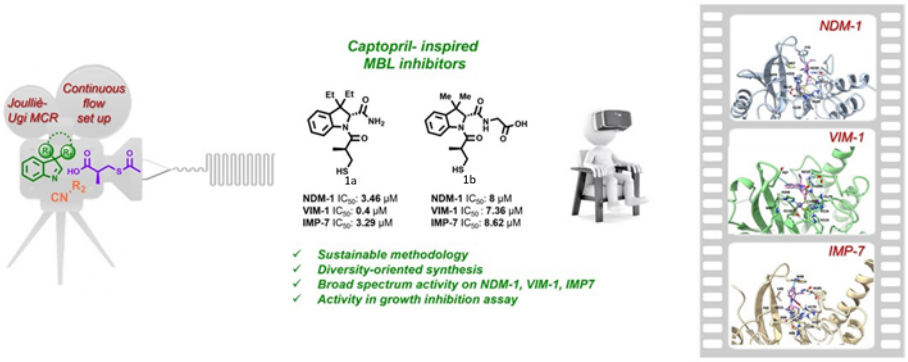

Figure 1: Novel captopril-inspired broad-spectrum MBL inhibitors

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AMYC BIOMED 2024

Cyclic Antimicrobial Peptides Analogues of Temporin L: Design and Synthesis of Novel Guanidine-based Derivatives

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Keywords: Amino acids, antimicrobial peptides, peptide synthesis

The increasing number of antibiotic-resistant bacterial strains has sustained the need to develop new antimicrobial agents. Antimicrobial peptides (AMPs) represent a viable alternative to classical antibiotics due to their properties.[1] Among them, temporins are cationic AMPs, derived from the frog skin secretions, and a notable isoform of this group is the 13-mer Temporin L (TL) peptide. TL exhibits a stronger and broader spectrum activity, but also a significant cell toxicity. It was designed and synthesized a library of macrocyclic peptide analogues characterized by non-canonical amino acids allowing different side-chain-to-side-chain crosslinking strategies (*e.g.*, lactam bridge, guanidino-bridge). In particular, the incorporation of these linkers is also expected to impact on the physico-chemical (polarity contribution) and protease resistance properties. Furthermore, the effect of a new positive charge by the guanidine group as a cyclic motif or amino acid side chain group at a key position (Figure 1), has led to peptides with increased antimicrobial activity.[2,3] The most promising analogues, especially against Gram-negative bacteria, have showed a reduced cytotoxicity against human keratinocytes. Additionally, some of these showed an increase in pharmacokinetic properties. Preliminary results of antimicrobial activity and cytotoxicity obtained for these derivatives will drive the development of further analogues whose bacterial membrane interaction properties and their potential against multi-drug resistant infectious diseases will be investigated.

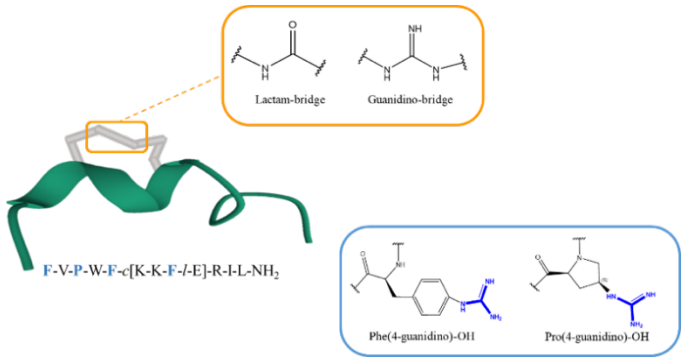

Figure 1. Structural modifications herein presented and applied to TL peptide sequence to improve antimicrobial properties.

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Sustainable Electroanalytical Platforms for the Continuous Monitoring of Chemical Parameters by Wearable Sensors

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Keywords: Sensors-Biosensors, Electroanalysis, Wearable, Platforms, Catecholamine

In recent years, the desire to enhance the quality of life has been generating the necessity to continuously monitor biophysical parameters, driving the widespread of wearable devices. While the use of physical wearable devices, which monitor heartbeat (ECG), blood oxygen and body temperature, has become commonplace, the development of wearable sensors for chemical parameters monitoring is still at an early stage. These wearable sensors must be flexible, thin, and comfortable while being mechanically stable and without signal drift. Furthermore, given the urgent need for sustainable consumption and production patterns, there is a growing interest in applying green processes in the manufacturing of electrochemical sensors,[1] simplifying at the same time the manufacturing steps of these devices.

In this study we applied sustainable materials and technologies to produce wearable electroanalytical platforms, using biodegradable and flexible polymers[2] and thin graphitic carbon electrodes. We investigated several techniques for creating conductive traces, including surface graphitization with a CO_2 laser [3], and graphene-based paper [4], to facilitate manufacturing and reduce costs. We tested the electrochemical performance of these innovative platforms through Cyclic Voltammetry (CV), Chronoamperometry (CA), and Electrochemical Impedance Spectroscopy (EIS). Their applicability as wearable amperometric sensors was evaluated by analyzing biomarkers of interest for the health control, like dopamine, ascorbic acid, and glucose.

All the investigated platforms showed interesting performance, confirming their potential application in electrochemical sensing. Their flexibility, thinness, low cost, and low environmental impact make them attractive candidates for the next generation of sustainable wearable sensors.

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BUG WARS: The Rise of G-Quadruplex

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Keywords: G-quadruplex, Groove Binders, Bacteria, Molecular Modelling, Biophysics

G-quadruplexes (GQs) are non-canonical four-stranded structural motifs formed by guanine-rich sequences identified in both DNA and RNA playing a wide range of pivotal biological functions in human and non-human cells. The most variable portions of the GQs are the loops-connecting the G-rich tracts, which can have different lengths and compositions[1].
Antimicrobial resistance (AMR) is an underrated problem that has been underestimated for decades promoted by the abuse and misuse of antibiotics. As a result, public health systems are facing enormous costs for the treatment of nosocomial infections due to AMR bacteria. In particular, the World Health Organization (WHO) has identified six highly virulent bacteria (E.S.K.A.P.E.) as the main causes of infections worldwide[2] [3].
In recent years, a few attempts were made to discover and develop new clinically relevant antibiotics mostly by developing close chemical derivatives of existing antimicrobial drugs, which represents a short-term solution as the AMR was already established. Hence, we are unable to efficiently treat multi-drugs resistance (MDR) bacterial infections and are unprepared for a potential AMR bacteria outbreak[4].
In this context, "the G-Q-eat ESKAPE" project aims to use a multidisciplinary approach combining computational models with biophysical tools to: *i*) identify new compounds able to interact within the groove of bacterial GQs for the treatment of microbial infections overcoming AMR; *ii*) develop a comprehensive platform for effective and rapid virtual screening as a first resource in emergency upon pathogen outbreak.

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pH-Responsive Multilayer Hydrogel Patches for Improved Burn Wound Healing

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Keywords: Burn Wound Healing, pH-Responsive Hydrogels, Alginate Hydrogel, Manuka Honey, Biocompatible Materials

Burn injuries are among the most severe and painful skin conditions, significantly affecting patients' quality of life and physiological functions [1]. Effective management of burns necessitates prompt treatment and innovative materials that facilitate efficient wound healing. Hydrogels, known for their high hydrophilicity, excellent biocompatibility, and ability to create an optimal environment for skin tissue regeneration, have emerged as promising therapeutic options [2-3]. This study introduces a novel protocol for fabricating pH-responsive multilayer hydrogel patches composed of biocompatible alginate (ALG) and bioactive agents like manuka honey (MH) with antibacterial properties. The hydrogel patches were crosslinked through ionic gelation with a calcium chloride solution. Their hydrophilicity was assessed by evaluating the swelling ratio, water content, and ability to absorb wound exudate to enhance healing and prevent infections. FTIR analysis provided insights into the chemical composition of the layers, while DSC analysis assessed thermal stability within the physiological range. Water vapor transmission rates (WVTR) were measured to determine the water vapor permeability of the patches. The pH-responsive degradation was also studied to confirm the patches' responsiveness to different pH levels. The prepared multilayer hydrogels showed high water content (>85%), high porosity, and good water vapor permeability, indicating their potential effectiveness for burn treatment. This research presents a promising advancement in burn care, offering an innovative approach to enhancing burn management and healing.

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Merging Electrochemical device and Deep Learning for FabryDisease Diagnosis at the point of care

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Keywords: Fabry Disease, Deep Learning, Screen-printed, Electrochemical Sensor

Fabry disease is a rare genetic disease of X-linked lysosomal accumulation. It results from mutations in the α-galactosidase A (GLA) gene, which encodes for α-galactosidase A (α-Gal A) enzyme activity that is absent or deficient in patients. This pathology causes the progressive accumulation of globotriaosylceramide (GL-3 or Gb3), its deacylated derivative, globotriaosylsphingosine (Lyso-Gb3), and related glycosphingolipids in plasma and tissue lysosomes throughout the body[1]. This lysosomal accumulation results in considerable damage to the heart, kidneys, and blood vessels that can be fatal[2]. Therefore, early diagnosis is critical for the management of Fabry disease and enables increased life expectancy of patients. This study aimed to develop an electrochemical sensor for the early diagnosis of Fabry disease using differential pulse voltammetry (DPV). A screen-printed electrode wasused to measure the presence of 4Methylubelliferyl-ad galactopyraniside, the substrate of the enzyme α-Gal A, in blood samples from healthy and diseased individuals. The results showed a higher peak intensity in blood samples from patients with Fabry disease than in healthy blood samples due to the presence of the substrate. To improve the accuracy of diagnosis, a Deep Learning algorithm was applied to classify the samples based on the shape of the DPV peaks. The algorithm used is Multilayer perceptron. The dataset consisted of 76 samples of healthy and 99 samples of Fabry patients. The test set was constructed with 20% of the healthy samples and 30% of the diseased samples, achieving 94% accuracy. This innovative approach combines the sensitivity of the electrochemical sensor with the effectiveness of machine learning, providing an early and accurate diagnosis of Fabry disease.

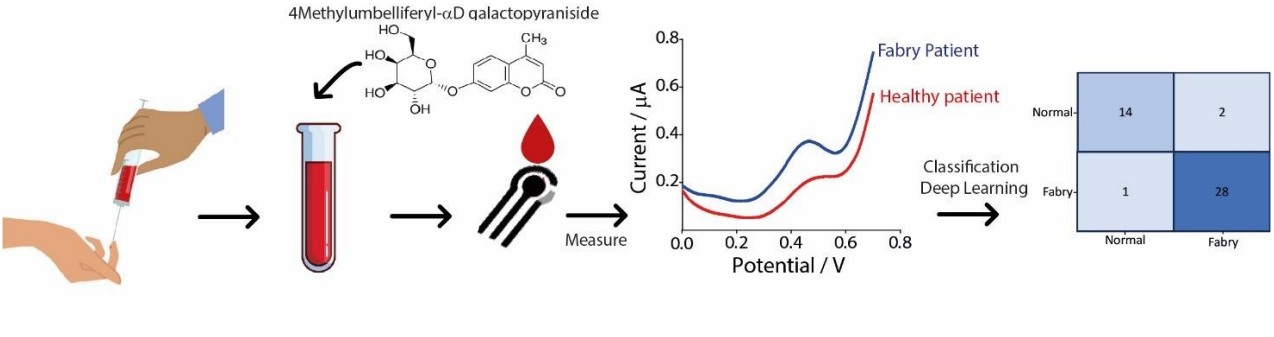

Fig. 1 Workflow from sampling to sample classification by deep learning.

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Hybrid nanoparticles based on mesoporous silica and functionalized biopolymers as drug carriers for chemotherapeutic agents

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Mesoporous silica nanoparticles (MSNs) are promising drug carriers for cancer therapy. Their functionalization with tissue/cell-specific targeting ligands and stimuli-responsive cap materials to seal drugs within the pores of MSNs is extensively studied for biomedical and pharmaceutical applications. In this work, MSNs were synthesized using a sol–gel strategy in the presence of a cationic surfactant as a template. Aqueous solution containing tetraethoxylmethane (TEOS) as a silica source and cetyltrimethylammonium bromide (CTAB) as a surfactant was used for the initial synthesis of MSNs. Subsequently, the obtained nanoparticles were subjected to calcination to completely remove the CTAB. Characterization of the nanoparticles was then performed using several analytical techniques, including low-angle X-ray diffraction (XRD) FTIR spectroscopy, thermogravimetric analysis (TGA), NMR spectroscopy, dynamic light scattering (DLS). Subsequently, the MSNs were coated with functionalized polymers such as hyaluronic acid esterified with folic acid (HA-FA) and chitosan esterified with dopamine (CS-DA) capable of facilitating their entry into tumor cells through receptor-mediated endocytosis and loaded with drugs with anti- tumor activity. The hybrid nanoparticles obtained were characterized and subjected to release studies in conditions that simulate the tumor environment.

Keywords: mesoporosus silicananoparticles, 5-fluoruracil, silymarin, hyaluronic acid-folic acid, chitosan-dopamine

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N-acylethanolamines act in innate and adaptive immune cells as Proresolving and immunomodulatory agents

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Keywords: Endocannabinoids, SPM, Anandamide, Resolution of inflammation, ALIAmides

N-Acylethanolamines are endogenous bioactive lipids that have been extensively studied for their immunomodulatory and pro-resolving properties across various diseases. Key members of this class include endocannabinoids (eCBs) such as arachidonoylethanolamide (AEA) and a variety of other eCB-like compounds commonly known as autacoid local injury antagonist amides (ALIAmides) [1-2]. Among these, adrenoylethanolamide (DEA) has not yet been studied as an anti-inflammatory agent and its role on immune cells such as lymphocytes is completely unknown. On the contrary, anandamide has been extensively studied in recent years as an anti-inflammatory agent due to its ability to reduce the production of pro-inflammatory cytokines and, in general, to act as an immunomodulatory agent that promotes return of homeostasis. However, despite these findings, its role in the resolution of inflammation – a process typically regulated by ω-3-derived lipids known as Specialized Pro-resolving Mediators (SPMs) that avoids deviant acute inflammation, remains underexplored. In this study, we investigated the pro-resolving and immunoregulatory properties of AEA on primary human monocyte-derived macrophages (MoDMs) and the effects of DEA on T cells. We discovered that AEA treatment at nanomolar concentrations boosts the production of specialized pro-resolving mediators (SPMs) such as Resolvin (Rv)D1 and RvE1 via the type 2 cannabinoid receptor (CB2), and enhances efferocytosis (the clearance of apoptotic cells), a key process in the resolution of inflammation, through CB2 and GPR18, without altering macrophage polarization and immunophenotype. Of note, GPR18, one of the main SPM receptors, can be activated by AEA [3], suggesting a direct role for this eCB in resolution processes. Additionally, our experiments showed that administering AEA or DEA reduces

the production of TNF-α, IFN-γ, and IL-17 in CD4+ and CD8+ T cells in a dose-dependent manner. Interestingly, these effects were inhibited by the CB1 inverse agonist SR141716A in CD8+ T cells, indicating a key role of this receptor in DEA signalling. Our findings demonstrate for the first time that DEA directly influences various T-cell subsets and provide evidence of AEA's role in modulating pro-resolving functions in human macrophages indicating that different lipid species participate in concert to achieve immune modulation.

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Molecular Cancer Imaging: Developing Novel PET Radiotracers Targeting the Human Epidermal Growth Factor Receptor HER2

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Keywords: Radiotracers, HER2, PET, Imaging, Cancer.

The human epidermal growth factor receptor 2 (HER2) has a long-standing history as a biomarker in cancer, playing a significant role in prognosis and treatment. Its oncogenic potential is primarily attributed to overexpression and/or gene amplification, driving tumor development and progression by influencing key cellular processes such as proliferation, migration, invasion, and survival[1]. However, variability in HER2 expression across tumors affects the effectiveness of anti-HER2 therapies, emphasizing the importance of precise HER2 status assessment. In this context, Positron Emission Tomography (PET) imaging with anti-HER2 radiotracers provides non-invasive, whole- body diagnostics for tumor detection and therapy monitoring. Recent advancements in HER2 imaging agents havemoved away from high molecular weight constructs, like monoclonal antibodies, towards smaller molecules such as tyrosine kinase inhibitors (TKIs), addressing challenges such as limited tumor penetration and slow clearance. This study focuses on modifying the promising TKI SPH5030[2] (Figure 1) to develop new imaging agents for HER2. SPH5030, highly selective and potent ligand toward HER2 (IC_{50}^{HER2}=3.51 nM), is currently under investigation in phase I clinical trials for HER2-amplified and HER2-mutant cancers. Two radiolabeling strategies are proposed: firstly, incorporating the positron-emitting radionuclide ^{11}C into the methyl group of SPH5030 generates **radiotracer 1** (Figure 1), structurally equivalent to SPH5030. Secondly, **radiotracer 2** (Figure 1) is synthesized by substituting the methyl group with a [^{18}F]fluoroethyl prosthetic group, utilizing the extended half-life of ^{18}F. Both tracers will be assessed for biodistribution, pharmacokinetics, and uptake in preclinical settings for HER2 imaging.

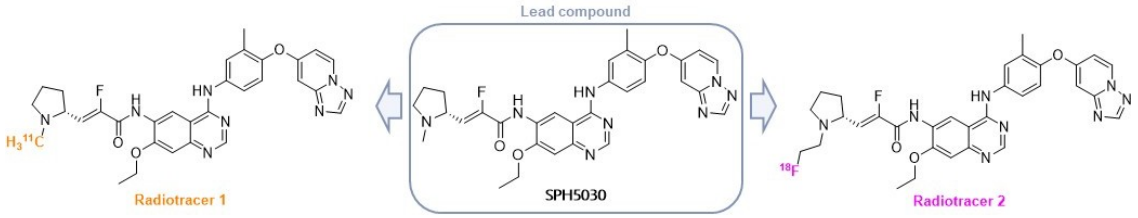

Figure 1: Development of PET tracers for HER2.

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Design, synthesis and characterization of gold-peptide bioconjugates for integrin targeted therapy of high malignant cancers

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Keywords: cancer, therapy, gold, peptide, integrin.

Cancer is one of the most widespread diseases in the world. Platinum-based drugs, like cisplatin, are extensively used as anticancer agents. [1] However, these drugs are of limited efficacy due to severe side effects, including toxicity and intrinsic or acquired resistance. These limitations prompt the design and synthesis of novel chemotherapeutic agents based on other metals, such as gold. Gold-based compounds have emerged as a promising alternative to Pt-based drugs since they show low toxicity and selective activity against specific cancer. [2] Many hypotheses have been formulated on the possible mechanism of action of gold compounds. Au-based drugs mainly act through the modification of selected enzymes, which leads to loss of function. [2,3] Unfortunately, they often show poor selectivity towards tumor cells, causing several side effects.
In order to exploit peptides as drug delivery systems, selective gold-peptide bioconjugates have been designed, synthesized and characterized.
In tumor cells, integrins expression contributes to tumor progression and metastasis by increasing tumor cell migration, invasion, proliferation and survival. [4] Among members of the integrin family, $\alpha v\beta 3$ can be considered a validated tumor marker since it has a relatively limited cellular distribution in normal tissues, but it is highly expressed in many solid tumors (e.g. melanoma). RGDechi is a very promising peptide able to discriminate, in vitro and in vivo, between $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins, and selectively modulate $\alpha v\beta 3$ function in some tumors. [5]
In this scenario in order to exploit the biological properties of RGDechi and the antitumor activity of the metal-based drugs attaining a synergic action, gold compounds [2] conjugated with RGDechi peptides were designed. [6,7]
The reaction conditions for obtaining gold-peptide bioconjugate have been investigated and optimized by using as starting reagent simple peptide sequence containing also RGD motif, to validate the reaction conditions. The c-fKRGD peptide, well known as $\alpha v\beta 3/\alpha v\beta 5$ antagonist, and its linear precursor have been selected as peptides bearing RGD sequence. [8]
Gold-peptide bioconjugates could be useful for the therapy of highly aggressive integrin-expressing tumors. Overall, potentially all solid tumors expressing moderate to high levels of $\alpha v\beta 3$ integrin can benefit from the new gold-peptide bioconjugate systems developed.
The project was funded by AIRC IG2020 (ID24528).

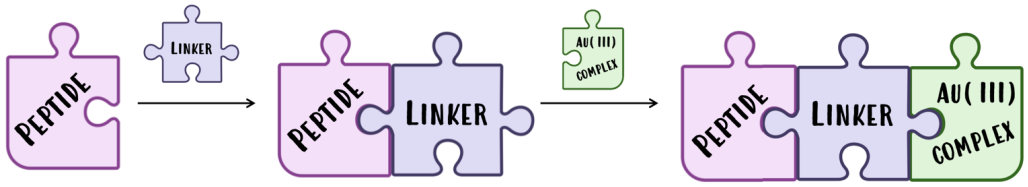

Figure 5 - Synthetic strategy toward Au(III)-peptide bioconjugates.

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Effect of air quality on oxidative stress and elemental levels in hair and urine of Italian and Chilean students

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Keywords: PM10; elemental analyis; ICP-MS; oxidative stress biomarkes

In this study, urinary oxidative stress biomarkers and the concentrations of 41 elements were measured in the hair and urine of students from two universities: one in Rome and one in Santiago, Chile. In addition to biological samples, PM_{10} samples were collected to analyze their elemental content and oxidative potential (OP; measured using cell-free DCFH, AA, and DTT assays). All elements were analyzed using ICP-MS and HG-AFS (1,2), while oxidative stress biomarkers were determined by HPLC/MS-MS. The final concentration of the analytes was expressed in µg/g of creatinine to normalize values with respect to urine dilution variability (3). During data processing, the influence of variables related to the participating students, such as age, sex, body mass index, smoking, and diet, was evaluated.
The average PM_{10} concentration was 26 µg m-3 in Rome and 71 µg m^{-3} in Santiago, Chile, indicating significantly different inhalation exposure levels in the two areas. Specifically, levels of Cu, Mo, Sn, and Sb—tracers of non-combustive vehicular traffic (mechanical abrasion of brakes; 4,5)—were significantly higher in Chilean PM10 samples compared to Italian ones. The OP values measured by the DCFH and AA assays were also notably higher at the Chilean site, confirming a significant contribution of non-combustive vehicle traffic to the oxidative potential of the dust. The different elemental concentrations in PM10 resulted in different metal and metalloid accumulation levels in the two biological matrices studied. The elements that showed the most significant differences between Italian and Chilean students in both biological matrices were Cs, Hg, Mg, Mo, Rb, Sr, Tl, and Zn. In contrast, Al and Sn varied significantly in urine and hair, not based on geographic area but depending on the participant's gender. Oxidative stress biomarkers showed higher values in the urine of smokers, while no significant differences were found between Italian and Chilean urine samples.

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AMYC BIOMED 2024

Targeting olfactory receptors for the treatment of lymphoma with new [1,2]oxazole based small molecules

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Keywords: Lymphomas, Olfactory receptors, OR13A1, [1,2]oxazole based molecules

Lymphomas are blood malignancies generated from lymphocytes, recurring among the ten most common cancers in developed countries. Although some progresses have been achieved along the years in increasing survival, many patients still succumb because of this disease. Hence, more efficacious approaches and better therapeutic options for refractory forms are needed. The G-protein-coupled receptors (GPCRs)play a key role in cellular physiology and homeostasis, and disruption of their pathways is associated with various diseases such as cancer. A new perspective in the treatment of lymphoma is the identification of ligands for the emerging subfamily of olfactory GPCRs (ORs), since ectopic ORs are highly expressed in different cancer tissues and their activation seems to affect cancer cell growth and progression[1]. Small molecules based on heterocyclic scaffolds constitute an important class of natural and synthetic products, widely used in the treatment of lymphoma in combination chemotherapy regimens. In particular overexpression, deletion or mutation of GPCRs are associated with the development of different types of Non-Hodgkin's lymphomas (NHL). Tricyclic [1,2]oxazole-based compounds**1** and **2**, previously investigated by us, showed potent growth inhibitory effect against four different lymphoma histotypes with GI_{50} values in the low micromolar-nanomolar range[2].The most sensitive cell line was found to be GCB-DLBCL (SUDHL10) expressing the GPCR olfactory receptor OR13A1. These results paved the way for the synthesis and biological evaluation of a new class of heterocyclic compounds targeting ORs. All new derivatives were tested with the glosensor luminescence assay in the Hana3A cell line over-expressing OR13A1.Compounds GSX2 and GSX20 emerged as an excellent ligand, showing nearly 2-fold stronger binding interaction with OR13A1 than the natural ligand cyclohexanone. On the basis of these results, iterative cycles of synthesis are ongoing to evaluate a structure-activity relationship (SAR) of the core structure with the aim to identify new candidates targeting ORs.

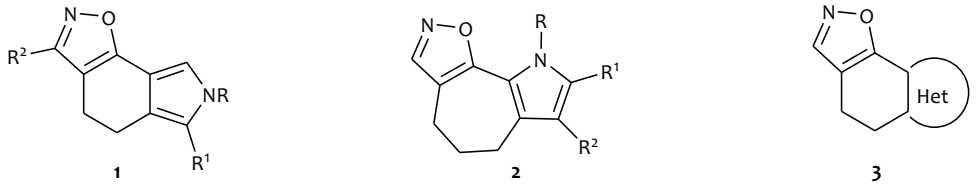

*Figure. [1,2]oxazolo[5,4-e]isoindoles***1**, *pyrrolo[2',3':3,4]cyclohepta[1,2-d][1,2]oxazoles***2**, *new tricyclic [1,2]oxazole-based compounds* **3**.

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Solid lipid nanoparticles encapsulating a benzoxanthene derivative modulate angiogenic parameters and inflammation in VEGF-stimulated angiogenesis in a model of human blood-brain barrier

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Keywords: SLN, benzo[*k,l*]xanthene lignans, VEGF-induced angiogenesis, blood-brain barrier.

Angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is a key process in biological, related to growth and repair. It is a finely regulated process, however, in some pathologies such as cancer, the regulatory mechanisms fail, and angiogenesis becomes pathological, therefore it has become a pharmacological target [1]. Antiangiogenic property is a characteristic of lignans, a secondary metabolite class of plants [2]. Due to its low aqueous solubility, a benzo(k,l)xanthene lignan derivative (BXL) (Figure 1) was encapsulated in solid lipid nanoparticles (SLN). Unloaded and BXL loaded SLN were prepared using Precirol® ATO 5, Gelucire 50/13 and Tween 80 and by the Phase inversion temperature method combined with Ultrasonication [3]. Unloaded and loaded SLN were characterized through photo correlation spectroscopy and calorimetric studies. Testes in a model of human blood-brain barrier were carried out. CCK-8, wound healing, IL-8 and PGE2 secretion assays were carried out on Human Brain Microvascular Endothelial Cells (HBMEC). Unloaded and BXL loaded SLN showed desirable values of dimensions (less than 200 nm), PDI (less than 0.2) and zeta potential (about -20 mV), indicating the stability of the samples. Loaded SLN showed an encapsulation efficiency of 61.5%. The in vitro release of BXL from SLN was carried out employing the dialysis method and showed that BXL was retained into the SLN for the time period studied. Calorimetric studies confirmed the encapsulation of BXL in the SLN matrix and the interaction of BXL loaded SLN with biomembrane models made of multilamellar vesicles. In VEGF-stimulated HBMECs (miming cancer-associated angiogenesis), both SNL and BXL loaded SNL induced cytotoxicity and inhibition of cell migration. Unlike SNL, BXL loaded SNL significantly reduced the in vitro tubulogenesis and the secretion of PGE2 and IL-8, mediators of VEGF-induced inflammation. Results suggest that the formulation represents a potential strategy for the adjunctive treatment of pathologies associated with aberrant angiogenesis.

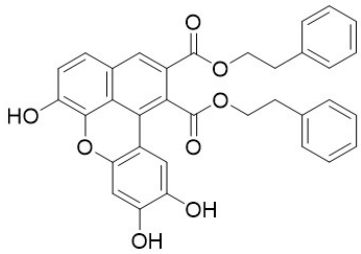

Figure 1. Chemical structure of benzo(k,l)xanthene lignan derivative (BXL)

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Paddlewheel diruthenium complexes *versus* amyloid-beta aggregation

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Keywords: Metallodrugs, amyloid aggregation, paddlewheel diruthenium complexes, amyloid-beta

Metallodrugs have attracted a great deal of interest in the field of neurological disorders due to their potential to effectively modulate the aggregation of amyloid proteins, a prominent feature of many neurodegenerative diseases. These conditions are characterized by the build-up of misfolded proteins, like amyloid-beta (Aβ) in Alzheimer's disease and the subsequent formation of toxic amyloid fibrils [1]. Due to their unique properties, metal complexes can inhibit the formation of large oligomers through direct and indirect interactions with amyloid peptides. Several studies have shown that ruthenium (Ru)-based complexes are an excellent choice due to their capability to inhibit the aggregation and toxicity of Aβ. Within the group of Ru-compounds, paddlewheel diruthenium complexes (Ru_2^{5+}) have emerged as source of inspiration for drug design, because they offer a novel plethora of valuable molecular scaffolds that are not possible with mononuclear compounds [2-3]. Herein, we report on the ability of two paddlewheel diruthenium compounds to act as aggregation modulators of $A\beta_{1-42}$ and its peculiar fragments $A\beta_{1-16}$ and $A\beta_{21-40}$ [4-5]. By means of different spectroscopic (e.g., fluorescence assays, UV-vis absorption, and electrospray ionization mass spectrometry) and microscopic (scanning electron microscopy and confocal microscopy) techniques and cellular assays, their mechanisms of action have been elucidated. The overall data may open new perspectives for the use of Ru_2-based compounds as novel drugs for neurodegenerative diseases.

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Unveiling the dominant enantiomer of 3MCPD esters usingIndirect Mass Spectrometry approaches based on GC-MS

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Keywords: 3MCPD, GC-MS, process contaminant, vegetable oils, chiral resolution.

[38] monochloro-1,2-propanediol (3MCPD) and its fatty acids esters are foodborne and thermally induced contaminants formed during the manufacturing process at high temperatures of vegetable oils and different foodstuffs [1]. In 2013, the International Agency for Research on Cancer classified 3MCPD as possibly carcinogenic [2], and the European Food Safety Authority published a scientific opinion on human health risks due to the presenceof 3MCPD fatty acid esters in food [3], updating the tolerable daily intake to 2 µg/kg bw per day in 2018 [4]. Interestingly, 3MCPD exists as two enantiomers that appear to exhibit different biological activities: R-3MCPD has demonstrated kidney toxicity, while S-3MCPD has shown an antifertility effect in males [5]. Currently, the health riskassessment is conducted using the concentration levels expressed as the sum of the two enantiomers, and little information is present in the literature about the enantiomeric composition of 3MCPD in food products.

We propose a combination of two indirect GC-MS methods designed to evaluate both the total content of 3MCPD esters after hydrolysis and the ratio between the two enantiomers. We analyzed 23 vegetable oils, 1 fish oil, and 3 margarines. We quantified 3MCPD esters within the limits established by the European Union in the range of 0.044to 1.435 µg/g. The two enantiomers, when detectable, were present in a 1:1 ratio.

Given these premises, if the two enantiomers can be separated and quantified, the health risk assessment can be revised and done specifically for the R-3MCPD and S-3MCPD. More studies on the toxicity of the two enantiomers are needed to determine the toxicity of each single enantiomer. In the future, we will apply these approaches to food samples as well to verify whether the ratio remains constant or changes under specific conditions.

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Development of a Chemiluminescent Readout for Type III CRISPR-Cas Systems: A Novel Nucleic Acid Detection Tool

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Keywords: Biosensor, Chemiluminescence, RNAzyme, CRISPR-Cas systems

CRISPR–Cas is an adaptive immune system in prokaryotes that provides sequence-specific immunity against mobile genetic elements (MGEs), such as phages, transposons and (conjugative) plasmids, although other non-immune functions have been identified as well [1]. More than a decade of research has highlighted the vast diversity of these systems, as indicated by their classification, which now identifies two main classes, six types, and many different subtypes. Type III CRISPR–Cas systems make use of a multi-subunit effector complex to target foreign (m)RNA transcripts complementary to the guide/CRISPR RNA (crRNA). Basepairing of the target RNA with specialized regions in the crRNA not only triggers target RNA cleavage, but also activates the characteristic Cas10 subunit and sets in motion a variety of catalytic activities that starts with the production of cyclic oligoadenylate (cOA) second messenger molecules from ATP. These messenger molecules can activate an extensive arsenal of ancillary effector proteins carrying the appropriate sensory domain: CARF or SAVED, generally fused to a wide range of catalytic domains [2].

Based on these characteristics type III CRISPR-Cas systems have a high potential for being repurposed as a novel, highly sensitive, robust nucleic acid detection tool. To exploit the signal amplification potentiality of this system, the production of the second messenger can be coupled to an easy read-out. A well-characterized example of a CARF-associated enzyme is a cOA-dependent nonspecific RNase Csx1. This enzyme was selected to establish a synthetic signal transduction route consisting of an RNA-targeting TtCmr/crRNA complex that generates cOA molecules, which in turn trigger the cleavage of a reporter RNA by Csx1 thereby generating a detectable fluorescence signal. Recently, this system has already been developed by a start-up and made commercially available for highly accurate diagnostics to the point of need [3]. However, the technique relies on a fluorescence method that requires a light source, making it difficult to miniaturize for the development of a portable analytical device.

To overcome this limitation, we explored the possibility to utilize the same type III CRIPRS-Cas-mediated activation of Csx1 RNAse technology for detecting nucleic acid sequences, employing a chemiluminescent (CL) readout for enhanced sensitivity, ease of use and amenability of miniaturization.

The proposed method relies on the cleavage of a specific G4 RNA probe sequence [4] by the Csx1 RNase, which is allosterically activated only in the presence of cOAs. The G4 RNA probe sequence has a G-quadruplex structure capable of exhibiting peroxidase-like activity in the presence of hemin, catalysing the chemiluminescent reaction between luminol and hydrogen peroxide. The Csx1 is activated only in the presence of the target RNA recognized by the CRISPR-Cas complex, leading to the degradation of the G4 RNA probe and resulting in the turn-off of the chemiluminescent signal.

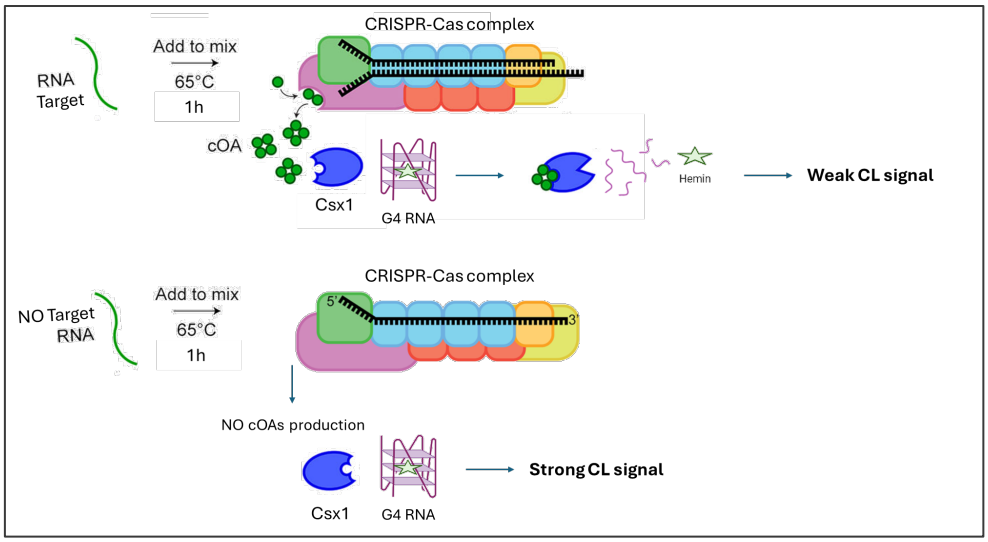

Figure 1. Mechanism of the method proposed.

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Ackowledgements:

We are grateful for the support from: Nano-ImmunoEra (European Union's MSCA Staff exchange Horizon Europe programme Grant Agreement Number 101086341); PRIN2017 project (Prot. 2017Y2PAB8); PRIN2017 project (Prot. 2017YER72K).

BIOFILM-DIRECTED MOLECULES TO OVERCOME ANTIBIOTIC RESISTANCE

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Keywords: antibiotic resistance, Gram-negative bacteria, quorum sensing, quinolones, benzimidazoles.

Bacterial biofilms are microbial communities encased in a matrix composed by extracellular polymeric substances and are found on biotic or abiotic surfaces. Albeit biofilms confer the bacteria a higher degree of survival in harsh environments, they however represent a threat for human health, since they affect antimicrobial efficacy and the immune response, contributing to antibiotic resistance.[1]
Among the diverse mechanisms involved in biofilm formation, Quorum Sensing plays a pivotal role: it consists of a complex regulation system governing the assembly of the structural components of biofilm, through the production of signaling molecules called autoinducers.[2] Based on this assumption, as a continuation of a previous research, we designed new compounds acting as QS antagonists with the aim of preventing biofilm formation or taking down already formed biofilm in different strains of Gram-negative bacteria.

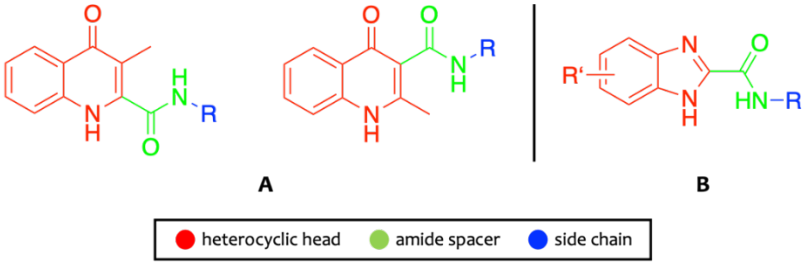

Figure 1. New heterocycles-based molecules acting against bacterial biofilm.

Our work consisted in the design of two series of compounds (A and B in figure 1) based on a heterocyclic core that was attached, through an amide spacer, to a side chain carrying moieties with different steric and electronic features. Specifically, compounds on series A were based on Quinolone derivatives, a group of potent autoinducers in *P. Aeruginosa*, while those in series B were derived from some benzimidazole hybrids employed as anti-QS agents.[3]
Preliminary *in vitro* tests were carried out on different strains of Gram-negative bacteria, such as *E. Coli* and *B. Cenocepacia*, in which some compounds showed a significant reduction of biofilm. Also, more in-depth studies have been planned to validate these early results on a wider number of bacterial strains.
The obtained results will constitute a promising starting point for further optimization of the anti-QS profile by defining the important structural requirements for this type of activity.

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A multi-analytical approach to study oxidative stress in bees

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Keywords: biomonitoring, oxidative stress biomarkers, environmental contaminants, HPLC-MS/MS, ICP-MS, landfill fire

Of all pollinating insects, bees are the most important group, though, due to their high sensitivity to environmental pollution [1], their global population is decreasing significantly [2]. Landfill fires are disasters of high environmental impact, for the vastity of contaminants, such as carbon monoxide and dioxide, nitrogen oxides, volatile organic compounds, persistent organic pollutants, aldehydes, and toxic or potentially toxic elements, that can be produced from waste combustion [3]. These contaminants can accumulate in bees and induce oxidative stress (4,5), this can lead to various pathologies and imparities, e.g., physical and cognitive deficits, that decrease beehive functionalities [1]. Aim of the present in-field study was the evaluation of elemental accumulation and oxidative stress levels in bees exposed to an incidental landfill fire, as well as the beneficial effect of the administration of *Quassia amara*, or probiotics, in reducing them.
An experimental apiary was placed nearby the landfill of Malagrotta (RM), central Italy (41°51'49.9 N 12°19'46.5 E), from April to July 2022, across a landfill fire occurred on 15th June 2022. The apiary, six beehives in total, was divided into three groups and fed with three different treated feedings (placebo, *Q. amara*, and probiotics). Monthly collected bees were analyzed for their elemental content by ICP-MS and CV-AFS [4,5], physiological oxidative stress and potential post-transcriptional damage, defined in terms of hydrogen peroxide and protein carbonyl group contents [5], and oxidative stress biomarkers by HPLC-MS/MS [6].
The results have shown that *Q. amara* and probiotics protect against the accumulation of toxic or potentially toxic elements. Indeed, compared to the placebo-fed group, a lower concentration of Ba, Be, Cd, Co, Fe, Li, Mn, Sn, Ti, and U was found in the *Q. amara*-fed group; and As, B, Ba, Cd, Co, Fe, Li, Mn, Ni, Pb, Sn, Ti, and U in the probiotic-fed group [5]. Both treatments also protected against oxidative stress: hydrogen peroxide content was lower than that of the control group during the study, and following the accident, the oxidative damage to proteins increased in the placebo group only. After the fire event, 5-methylcytidine (5-MeCyt), an epigenetic marker of RNA damage, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), a RNA oxidative stress biomarker, and 3-nitrotyrosine (3-NO2Tyr), a protein oxidation biomarker, increased in bees.
The present in-field study provided new knowledge on the effects of environmental pollution on bees' health and possible effective measures to protect this important insect.

We acknowledge the Ph.D. programs on green topics, PON Research and Innovation 2014–2020 project, funded by FSE REACT-EU

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Real-time fluorescence tracking of the *in vitro* degradation of a vascular graft

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Keywords: medical device validation, degradation, fluorescence, vascular graft, in vitro

Introduction: To design a biodegradable medical device that is functional but, most of all, safe, an in-depth understanding of its degradation kinetic is needed. Several attempts have been made to obtain real-time monitoring of the device degradation through magnetic resonance imaging, X-ray computed tomography, and fluorescence imaging [1]. Among those, the fluorescence-based tracking method received the most attention since it can be used *in vitro* and *in vivo* without active contrast agents or complicated instrumentation [2]. After fluorescently labeling the material, its degradation can be tracked and quantified through fluorescence measurements. In our case, fluorescence analysis was coupled with protein quantification to track *in vitro* the degradation of the Silkothane® Arteriovenous Graft fabricated by Dialybrid that comprises the biodegradable and labeled silk fibroin (SF) and the non-degradable polyurethane [3].

Methods: 6 cm-long grafts were soaked into 0.1 M sodium carbonate-bicarbonate buffer (pH 9) with a fluorescein isothiocyanate (FITC) (Merck Life Science, Milan) concentration of 50 µg/mL, kept overnight at 4 °C. Then, the samples were washed twice (4 hrs and overnight) under shaking in MilliQ water. A labeled graft was incubated at 37 °C with Protease XIV (Merck Life Science, Milan) dissolved in 40 mL of PBS at a final concentration of 0.05 mg/mL. A stained control specimen was incubated with the buffer alone in the same conditions. Periodic sampling from both buffers was performed at 0.5, 1, 2, 4, and 8 hrs. Protein quantification using Bicinchoninic acid assay (BCA), and fluorescence measurements were performed. SF concentration was calculated by subtracting the enzyme contribution. Finally, the percentage cumulative release was determined for proteins and FITC [4]. The experiment was repeated twice.

Results: As expected, SF was released only by the digested graft during the time frame considered (Figure 1.a). Moreover, the amount of fluorescent probe released from the digested samples differed significantly from that eluted from the undigested one (Figure 1.b), meaning that a fluorescence signal can be related to the weight loss induced by enzymatic digestion. Finally, the cumulative release of SF and FITC showed similar trends, suggesting that tracking the fluorescence over time enables the prediction of graft degradation kinetics (Figure 1.c).

Conclusion: Through the methodology proposed hereabove, we could covalently and specifically label the SF in the graft and track the release of FITC-labeled SF induced by enzymatic digestion. These results allowed the validation of fluorescent SF labeling to characterize the graft's degradation kinetic *in vitro*. Moreover, although the protocol was optimized specifically for the Dialybrid product, the methodology developed can be conveniently adapted to other protein-based medical devices.

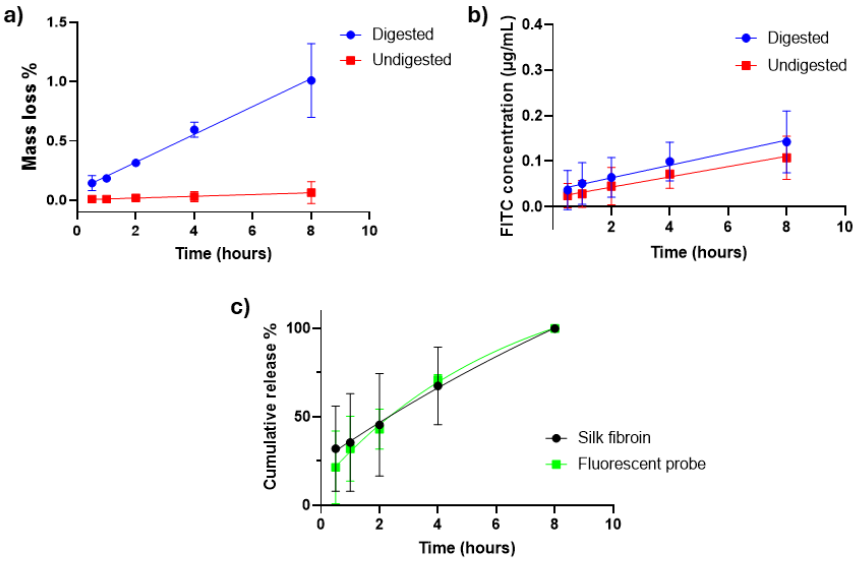

Figure 6: Graphs depicting a) the percentage mass loss and b) the measured FITC concentration in the buffers of digested and undigested samples over time. Graph c) shows the percentage of cumulative SF and FITC released from digested samples.

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Characterization of human calcium binding protein CIB2 in solution NMR

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Keywords: NMR structure characterization; Alphafold2; EF-hand; Calcium -and Integrine-Binding protein 2

Calcium- and Integrin-Binding protein 2 (CIB2) is a small protein with a role in mediating Ca(II) signaling. It belongs to a family of calcium and integrin-binding proteins containing four EF-hand domains that change conformation upon Mg(II) and/or Ca(II) binding[1]. Its broad expression levels in a variety of tissues suggests its involvement in a multiplicity of physiological and disease-associated processes[2]. Previous biochemical and biophysical characterization of CIB2 showed that it does not work as a calcium sensor but it is found in a Mg(II)-bound form under physiological conditions[3]. Anyway, at variance with the other proteins of this family, an atomic level characterization of CIB2 in the presence of both Ca(II) and Mg(II) ions is missing. Based on a combination of solution NMR and isothermal calorimetry experiments, we characterize the interaction of CIB2 with both Ca(II) and Mg(II) ions and the protein regions involved in the interaction with the α7B integrin target. Analysis of experimental amide nitrogen (^{15}N) relaxation rates (R_1, R_2, and ^{15}N-{^{1}H} NOE), combined with molecular dynamic simulation, show that EF-4 domain display mobility regardless of the specific metal ion bound in the binding site and demonstrate that the Ca(II) and Mg(II)- bound state is relatively floppy with pico-nanosecond motions induced in a region responsible for target recognition. These results offer novel insights into the dynamic regulation of target recognition and unravels the role of metal binding events in CIB2.

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Origami-Shaped Chemosensor for Portable Total Antioxidant Capacity (TAC) Assessment employing MOF-catalyzed Chemiluminescence

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Keywords: MOF, Chemiluminescence, Antioxidant, Sensor.

Over the past decade, interest in the field of porous reticular materials, especially metal-organic frameworks (MOFs), has grown greatly due to their outstanding performance, which have led to broad applications including separation, catalysis, energy storage, sensing and biomedicine [1].
In recent years, it has been discovered that MOFs can be used as effective catalysts to significantly increase the chemiluminescent (CL) emission of a luminol-H_2O_2 system in a basic environment. Among these catalytic materials, MIL-101 (Fe) has been shown to possess peroxidase-like activity in the presence of luminol and hydrogen peroxide [2].
Microfluidic paper-based analytical devices (µPADs) have recently attracted much attention due to their ease of use, low consumption of reagents, low cost, rapidity, portability, and disposability. These devices use paper as a substrate to create microfluidic structures (e.g., channels, reagent mixers, reaction chambers) by patterning hydrophobic materials on hydrophilic paper. Moreover, the use of *origami* (paper folding) techniques in the fabrication of µPADs has given researchers the opportunity to fabricate 3D paper-based devices, which provide high flexibility of application and allow for conducting complex multistep analytical procedures. The combination of PADs with portable device-based CL detection technology has promoted the development of miniaturized and intelligent CL detection platforms for point-of-care testing and on-site analysis [3].
Over the past few years, there has been a continuously growing interest in antioxidants by both consumers and the food industry. The beneficial health effects of antioxidants have led to their widespread use in fortified functional foods, as dietary supplements, and as preservatives. A variety of analytical methods are available to evaluate the total antioxidant capacity (TAC) of food extracts and beverages. However, most of them are expensive, time-consuming, and require laboratory instrumentation. Therefore, simple, cheap, and fast portable sensors for point-of-need measurement of antioxidants in food samples are required [4].
Here we describe an *origami*-shaped chemosensor for in situ TAC assessment of real matrices by exploiting the CL delay induced by the presence of antioxidant substances in the sample under analysis. The *origami* µPAD was produced by wax printing on chromatography paper. The reaction takes place on a ready-to-use analytical paper support where MOFs (MIL-101 Fe), luminol, and sodium perborate were previously adsorbed on different paper sheets and is monitored by using a portable Charge Coupled Device (CCD) camera. As shown in Figure 1, as the antioxidant power increases, the turn-on delay of the chemiluminescent emission increases, thus allowing the quantification of TAC in the matrix under investigation.

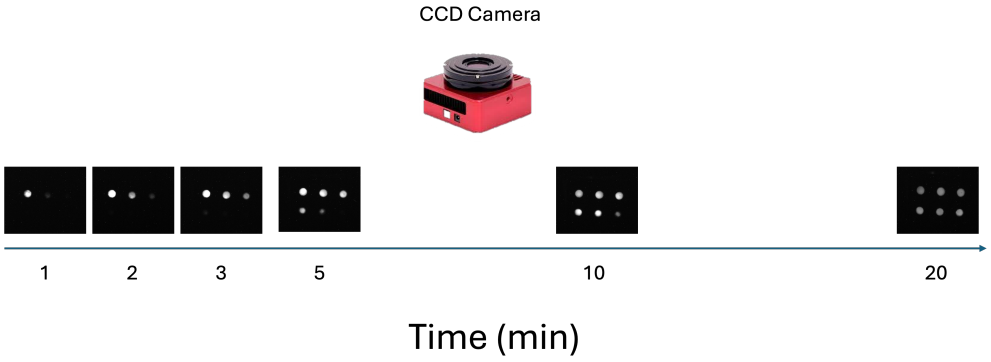

Figure 7. CL Signal obtained over time testing different concentration of gallic acid using a CCD camera

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Enhancing the Sensitivity of A375MM Melanoma Cells to Canonical Anti-neoplastic Drugs through G-Quadruplex Binders

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Keywords: Cancer, chemotherapeutics, G-quadruplex structures, G-quadruplex binders, synergism.

Chemotherapy represents the cornerstone of cancer therapy, involving the use of drugs that target rapidly dividing cells. Traditional chemotherapeutics such as alkylating agents, anti-metabolites, and plant alkaloids have proven effective in cancer treatment. However, they often come with severe side effects that can impact both the physical and mental well-being of patients.

In this context, sensitization of cancer cells to standard chemotherapy using small molecules is emerging as a promising approach to minimize chemotherapy-induced adverse effects and to try to improve the clinical outcomes [1,2].

Herein, exploiting the role of DNA G-quadruplex (G4) structures as anti-cancer targets [3], we propose the use of G4 binders to enhance the sensitivity of cancer cells towards traditional chemotherapeutics, potentially allowing to reduce their doses and, hopefully, their toxicity and adverse effects.

G4s fall among the most widely investigated non-canonical DNA secondary structures. They occur in guanine-rich sequences and consist of stacked layers of G-tetrads, planar arrangements of four guanines held together by eight Hoogsteen hydrogen bonds. These structures are highly prevalent in biologically relevant genomic regions (*i.e.*, telomeres and oncogene promoters), thus being potentially involved in the regulation of critical cellular processes, including telomere homeostasis and gene transcription [4]. In this regard, several lines of evidence have demonstrated that G4 formation/stabilization can impair telomerase activity or reduce oncogene transcription efficiency [5]. As a result, thousands of G4 ligands have been developed over the years, as potential anti-cancer tools [6].

In the present investigation, we exposed A375MM melanoma cells to a variety of conventional chemotherapeutics (Azacytidine, Cisplatin, Doxorubicin, 5-Fluorouracil, Methotrexate, Paclitaxel, and Vincristine) in combination with three well-established G4 ligands (RHPS4, Pyridostatin, and Berberine), using either simultaneous or sequential dosing schedules. Interestingly, the G-quadruplex binders could synergize most of the investigated anti-cancer drugs, with the entity of synergism being strictly dependent on both the treatment schedule and the drug sequence employed. Furthermore, the toxicity of the combinations demonstrating strong synergism was also tested on non-tumorigenic human keratinocytes (HaCaT), showing to be less pronounced than that on A375MM cancer cells, in most cases. Strikingly, immunofluorescence experiments highlighted the potential implication of G4 structures in the molecular mechanisms driving the synergistic interaction that we found between chemotherapeutics and G-quadruplex binders.

Overall, our findings might support the use of G4-interacting molecules to enhance the efficacy of standard anti-neoplastic drugs, significantly reducing their required doses.

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β-Cyclodextrin polymers to enhance the antibacterial activity of doxycycline and chloramphenicol

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Keywords: cyclodextrin, polymers, antibacterial, doxycycline, chloramphenicol

Nowadays, the clinical pipeline lacks quality new antibiotics due to costly and lengthy development [1].
For this reason, pharmaceutical industries focus research on delivery systems to able to enhance the effectiveness of the existing antimicrobials and to elude resistance mechanisms [2]. This interest has led to the exploitation of various β-cyclodextrin polymers and their conjugates as potential adjuvants and carriers for the poorly water-soluble drugs doxycycline and chloramphenicol (Fig. 1).
The **choice** to use cyclodextrin-based polymers is due to their ability to enhance the loading and bioavailability of insoluble drugs [3]. Moreover, they could modulate the antibiotic release thanks to their **controlled** characteristics [4].
The inclusion complexes of cyclodextrin polymers with the two antibiotics have been characterized using NMR and UV spectroscopy, demonstrating an enhancement of the drug solubility. The polymers were studied *in vitro*, both alone and in combination with doxycycline or chloramphenicol, using 1:1 and 1:2 molar ratios antibiotics/polymers, against two bacterial strains, *Escherichia coli* ATCC 9637 and *Staphylococcus aureus* ATCC 29213.
When tested with doxycycline, the neutral β-cyclodextrin polymer enhanced the antibiotic efficacy against both bacterial strains. For chloramphenicol, an increase in antibacterial activity was observed when combined with the β-cyclodextrin polymer functionalized with quaternary ammonium groups on both bacterial strains.
Our results indicate that the inclusion complexes of doxycycline and chloramphenicol in β-cyclodextrin polymers are a promising strategy to develop new formulations of these drugs, potentially enhancing their solubility and antibacterial efficacy.

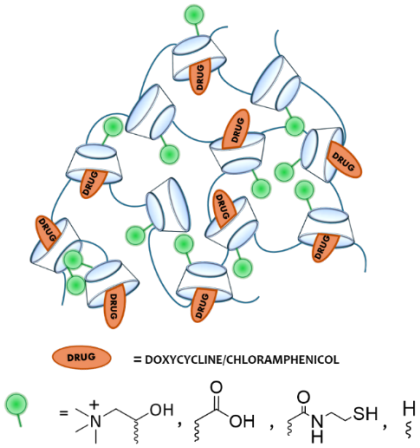

Figure 1. Polymers with antibiotics

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Hybrid microgels as novel delivery systems for active compounds

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Keywords: hybrid microgel; PNIPAM; keratin; caffeic acid

Abstract

The combination of the synthetic polymer poly(N-isopropylacrylamide) (PNIPAM), with natural biomolecule keratin, shows potential in the field of biomedicine, since these hybrids merge the thermoresponsive properties of PNIPAM with the bioactive characteristics of keratin[1,2].

In this study, we exploit keratin derived from wool waste in the textile industry, to synthesize hybrids with PNIPAM microgel with different synthetic approaches and keratin content, resulting in two formulations (HYB-P and HYB-M). Dynamic light scattering (DLS) and transmission electron microscopic (TEM) analyses indicated the formation of colloidal systems with particle sizes of around 110 nm for HYB-P and 518 nm for HYB-M. The temperature-responsiveness characteristic of PNIPAM microgels is retained in the hybrids, which have a temperature of volume phase transition very similar to the former (approximately 32.5°C), indicating that the presence of keratin does not influence the transition temperature [3].

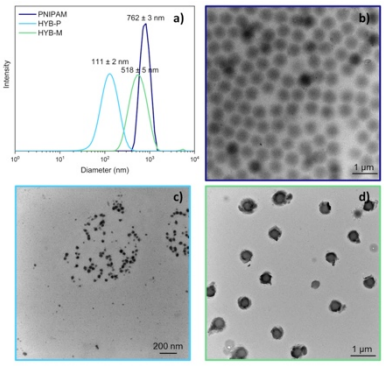

Figure 8: Dimensional distribution of microgels as determined by DLS analysis (a) and representative TEM micrographs of sole PNIPAM (b), HYP-P (c) and HYB-M (d) [3].

With the aim of considering the microgels as novel delivery systems for active compounds, caffeic acid has been selected as model drug. The influence of the encapsulated drug on size distribution was investigated by DLS, while the drug entrapment efficiency and the diffusion profile were assessed by UV-Vis spectrophotometer.

These hybrids exhibit unique structural characteristics and responsiveness to temperature and even to pH variation, suggesting their potential for various biomedical applications, as well as for the delivery of active compounds.

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Fecal biomarker discovery by mass spectrometry-based proteomics: a perspective towards non-invasive colorectal cancer screening methods

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Keywords: Mass spectrometry, Proteomics, Colorectal Cancer, Stool samples, Non-invasive screening

Colorectal cancer (CRC) represents the second-leading cause of cancer death worldwide.[1] Up to now, screening programs involve the detection of occult blood in stools - characterized by high false-positive rate and, in case of positivity, subsequent colonoscopy, whose invasiveness causes reluctance to perform it. Consequently, non-invasive diagnostic screening tests are strongly required to increase patient engagement, and to improve the prognosis and the efficacy of the pharmacological therapy. Mass spectrometry (MS)-based proteomics could serve as a powerful tool for discovering new disease biomarkers, to further develop reliable diagnostic tests for early and accurate diagnosis.[2,3] Investigation of non-invasive stool samples is crucial to boost population engagement in preventive care screening.

Hence, the present work aims to combine the discovery of faecal CRC biomarkers by MS-based proteomics, with the perspective of implementation of immunochemical point-of-care assays based on smart electrochemical sensing devices. Stool samples were collected from patients who underwent colonoscopy and had at least one adenomatous polyp or cancer, stratified according to histology in low-grade dysplasia, high-grade dysplasia, and adenocarcinoma; the study included also healthy controls. Untargeted MS-based shotgun proteomics was performed using a nano-LC coupled to an Orbitrap Exploris 480 with a High-Field Asymmetric Waveform Ion Mobility Spectrometry System. The applied sample preparation procedure involved the use of a lysis buffer combined with sonication and protein precipitation, and permitted to extract an average of 100 proteins per sample. A total of 513 distinct proteins were identified and semi-quantified across all the samples. Statistical analysis highlighted the presence of some enriched human proteins in patients with low-grade dysplasia, high-grade dysplasia and adenocarcinoma with respect to healthy subjects: two most promising CRC biomarkers were shortlisted, here generically named as PROT1 and PROT2 (**Figure 1**). The upregulation of these proteins throughout cancer progression found confirmation in the literature.

The next step of the project will be the development of electrochemical immunosensors for the non-invasive determination of PROT1 and PROT2 in stool samples, through proper optimization of experimental conditions, among which a fast and non-denaturant extraction procedure.

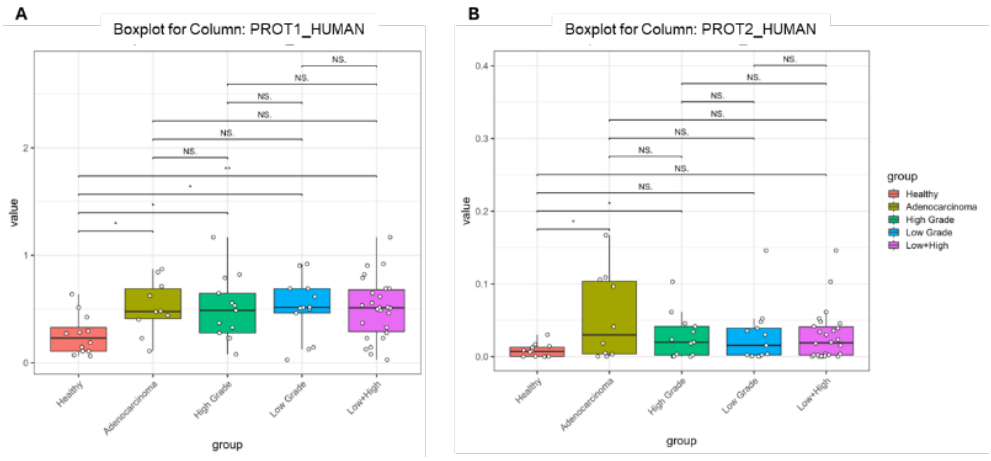

Figure 1. Box plot showing **(A)** PROT1 and **(B)** PROT2 regulation between healthy control patients and patients with different pathological states (adenocarcinoma, high-grade dysplasia, low-grade dysplasia). * p<0.05

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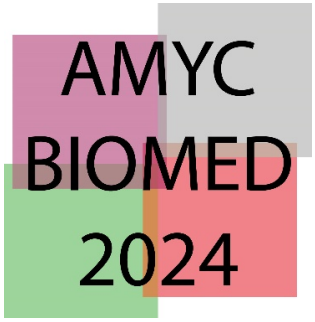
Acknowledgements: PRIN 2022 project N. 2022E8YHB7 (OmicSensing).



POSTERS

AMYC BIOMED 2024

PROBING THE EFFECTS OF CHEMICAL MODIFICATIONS ON THROMBIN BINDING APTAMER BIOLOGICAL ACTIVITY.

Carla Aliberti, Antonella Virgilio, Daniela Benigno, Ivana Bello, Valentina Vellecco, Elisabetta Panza, Mariarosaria Bucci, Veronica Esposito* and Aldo Galeone.

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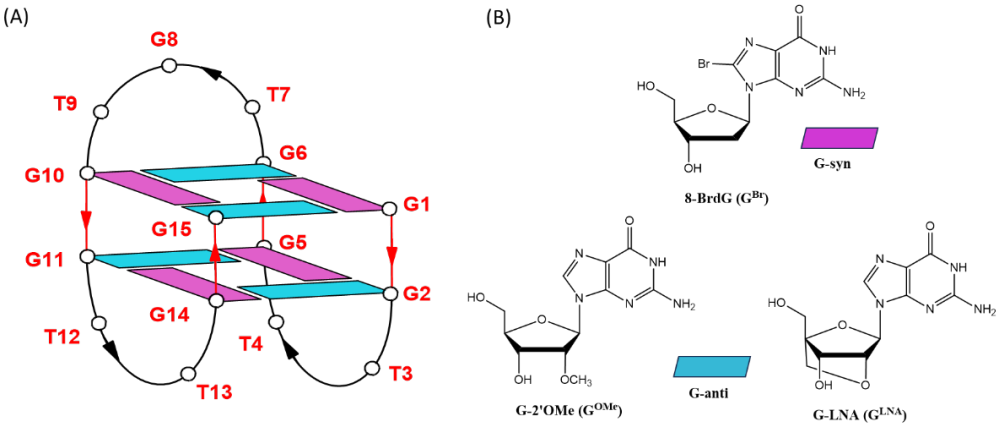
Keywords: G-quadruplex; thrombin binding aptamer; 8-bromo-2′-deoxyguanosine; locked nucleic acid guanosine; 2'-O-methylguanosine; antiproliferative activity.

Thrombin binding aptamer (TBA) is one of the best-known G-quadruplex (G4)-forming aptamers that efficiently binds to thrombin resulting in anticoagulant effect [1]. TBA also possesses promising antiproliferative properties. As with most therapeutic oligonucleotides, chemical modifications are critical for therapeutic applications to improve thermodynamic stability, resistance in biological environment and target affinity [2]. To evaluate the effects of nucleobase and/or sugar moiety chemical modification, five TBA analogues have been designed and synthesized preserving its chair-like G-quadruplex structure [3], being crucial for its biological activity, and their structural and biological properties have been investigated by Circular Dichroism, Nuclear Magnetic Resonance, non-denaturing PAGE techniques, and PT and MTT assays. The analogue **TBAB** contains 8-bromo-2′-deoxyguanosine (G^{Br}) in G-syn glycosidic positions, while **TBAL** and **TBAM** contain also locked nucleic acid guanosine (G^{LNA}) or 2'-O-methylguanosine (G^{OMe}) in G-anti positions, respectively. In **TBABL** and **TBABM** two modifications have been introduced with the aim to obtain synergistic effects. In fact, both contain 8-bromo-2′-deoxyguanosine (G^{Br}) in syn positions, while in positions adopting an anti-glycosidic conformation exhibit in turn locked nucleic acid guanosine (G^{LNA}) and 2'-O-methylguanosine (G^{OMe}). The most interesting results have been obtained for **TBAB**, which revealed an extraordinary thermal stability (Tm approximately 30◦C higher than that of TBA) and an anticoagulant activity higher than original aptamer. In the case of **TBABM** a promising cytotoxic activity against breast and prostate cancer cell lines were observed. These data indicate **TBAB** as the best TBA anticoagulant analogue here investigated, since some of the main limitations to therapeutic applications of this aptamer were overcome, and **TBABM** as a promising antiproliferative derivative.



Schematic representation of the TBA G-quadruplex. Deoxyguanosines in *syn* and *anti* glycosidic conformations are in purple and light blue, respectively (A); chemical structures of 8-bromo-2′-deoxyguanosine (G^{Br}), 2'-O-methylguanosine (G^{OMe}), and locked nucleic acid guanosine (G^{LNA}) (B).

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ZnO-on-cellulose hybrids in action: from experimental design to bacterial decontamination

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Keywords: Central composite design, ZnO synthesis, photocatalysis, cellulose.

Design of experiments (DOE) allows for improving the synthesis parameters of a large variety of functional materials, by determining a model to predict synthesis outcomes and the potential interactions among the synthesis variables, thus reducing process variability, time, number of experiments, and cost. To the best of our knowledge, the application of DOE to ZnO materials is quite limited, mainly because of the initial sub-optimal synthesis conditions. For instance, the seminal work from the group of prof. Wang reporting the "pick-the-winner rule" and "one-pair-at-a-time" analysis only allows for sequentially identifying optimal reaction settings [1], without understanding the effects due to interactions between the parameters. Recent works included DOE for producing ZnO for dyes absorption in aqueous solutions but without producing a surface response [2]. In this work, we use a central composite approach, based on a two-levels (-1; +1) design in addition to a central (0) point to test synthesis reproducibility. The design is based on a previously optimized synthesis protocol for electronic interfaces [3] and aims at understanding the effects of three parameters – i.e. Zinc precursor concentration, reaction time and KCl concentration, observing the effects on reaction yield and photodegradation efficiency of methylene blue dye. The synthesized ZnO are also characterized by ζ-potential and SEM to study their morphology. Whereas precursor concentration mainly affects reaction yield, KCl allows for increasing the (002) plane stabilization. Differently from our previous work based on ZnO/cellulose acetate composites [4], the synthesized ZnO is here absorbed (1 hour, 90 °C) onto precut ethyl cellulose or cellulose pads permitting the rapid release of ZnO when the ZnO-cellulose hybrid is soaked into aqueous solution. The photocatalysis experiments allow for the quantification of role of the three selected synthesis parameters, finally permitting to obtain a surface response. Finally, antimicrobial tests show the effective bactericidal ability of the synthesized materials on both gram-negative and gram-positive strains.

Acknowledgements:
The Italian Ministry of University and Research is acknowledged for funding through the PRIN 2022 project "2022WZK874 - Smart biopolymeric ZnO Nanowires composites for enhanced antibacterial activity", PNRR M4.C2.1.1, CUP: B53D23015730006 and Network 4 Energy Sustainable Transition – NEST", Spoke 1, Project code PE0000021, CUP B73C22001280006. The Advanced Technologies Network (ATeN) Center is acknowledged for hospitality and service.

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Determination of essential and toxic elements in herbal teas

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Keywords: analytical method; *Cannabis sativa L.*; sample preparation; inductively coupled plasma mass spectrometry

Recently, especially among young people, herbal teas have become increasingly common, often associated with specific health benefits, including anti-inflammatory, antioxidant, and slimming effects [1]. Numerous studies link tea consumption to the prevention of many types of cancer, diabetes, and Alzheimer's disease, as well as a boost in immune defenses and a reduction in inflammation and blood cholesterol levels [1]. In Italy, there still needs to be adequate legislation governing the trade, use, and content of chemical contaminants in herbal teas. In particular, some formulations of herbal teas, also due to the introduction of new ingredients such as algae and hemp, can be rich in chemical substances, including toxic metals, which can have various negative effects on the human organism, especially if we consider people in particular conditions, such as pregnant women, breastfeeding women, infancy and the elderly. For example, Al, As, Cd, Cu, Hg, and Pb can be present naturally in soil and water or released due to anthropogenic activities such as mining, agriculture, or industrial processes [2].
Furthermore, the regular intake of herbal teas can significantly contribute to exceeding the recommended daily intake (RDA) limits of some essential elements (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, and Zn) [3,4]. Hence, there is a need to develop reliable, rapid, and economical analytical methods for determining the elements in these products and carrying out a correct risk estimate, which is necessary to support decision-making processes to protect consumer health. The general objective was to carry out an estimate of the risks associated with the presence of 48 elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Fe, Ga, Hg, K, La, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, Sb, Se, Si, Sn, Sr, Tb, Te, Th, Ti, Tl, U, V, W, Y, Zn, and Zr) in different types of herbal teas identified among those most used in Italy. For this purpose, an analytical methodology for elemental analysis by inductively coupled plasma spectroscopy was developed. The best sample preparation procedure was evaluated using different reagent mixtures and four food standard reference materials (NIST1515, NIST1547, INCT-TL-1, and BCR482). The use of a 3 mL reagent mixture consisting of HNO_3 and H_2O_2, 2:1 (v/v) was found to produce satisfactory results in all cases. This method can be used for routine analysis and food quality control applications.

Acknowledgements: This work was funded by the project 2021 RM12117A5D872C39 (Principal Investigator M.L. Astolfi) financed by Sapienza University of Rome.

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Multiple drug-delivery strategies to enhance the pharmacological and toxicological properties of Mefenamic acid

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Keywords: Galactosylated prodrugs, Poly (glycerol adipate), Polymer-drug solid dispersion, Ulcerogenicity, Cytotoxicity.

To improve the biological and toxicological properties of Mefenamic acid (MA), the galactosylated prodrug of MA named MefeGAL [1] was included in polymeric solid dispersions (PSs) composed of poly(glycerol adipate) (PGA) and Pluronic® F68 (MefeGAL-PS). MefeGAL-PS was compared with polymeric solid formulations of MA (MA-PS) or a mixture of equal ratio of MefeGAL/MA (Mix-PS).
The *in-vitro* and *in-vivo* pharmacological and toxicological profiles of PSs have been investigated. In detail, we evaluated the anti-inflammatory (carrageenan-induced paw edema test), analgesic (acetic acid induced writhing test) and ulcerogenic activity in mice after oral treatment. Additionally, the antiproliferative activity of PSs was assessed on in vitro models of colorectal and non-small cell lung cancer.
When the PSs were resuspended in water, MefeGAL's, MA's and their mixture's apparent solubilities improved due to the interaction with the polymeric formulation. By comparing the *in-vivo* biological performance of MefeGAL-PS with that of MA, MefeGAL and MA-PS, it was seen that MefeGAL-PS exhibited the same sustained and delayed analgesic and anti-inflammatory profile as MefeGAL but did not cause gastrointestinal irritation. The pharmacological effect of Mix-PS was present from the first hours after administration, lasting about 44 hours with only slight gastric mucosa irritation. *In-vitro* evaluation indicated that Mix-PS had statistically significant higher cytotoxicity than MA-PS and MefeGAL-PS.
These preliminary data [2] are promising evidence that the galactosylated prodrug approach in tandem with a polymer-drug solid dispersion formulation strategy could represent a new drug delivery route to improve the solubility and biological activity of NSAIDs.

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A New Biocompatible Butyric Acid-Releasing Glucosamine Derivative for Transdermal Delivery

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Keywords: D-glucosamine, Butyric acid, Carrier Prodrug, Permeability study, Biocompatibility.

The carrier prodrug approach is a medicinal chemistry strategy adopted to refine the physicochemical and biopharmaceutical properties of parent drugs [1-2].
In the present work, this strategy was applied to improve the transdermal absorption of butyric acid (BA) by using a natural and nontoxic molecule, D-glucosamine (*N*-Glc), as a carrier. Accordingly, the design and synthesis of a new carrier prodrug, *N*-glucosamine tetrabutyrate (3-amino-6-((butyryloxy)methyl)tetrahydro-2H-pyran-2,4,5-triyl tributyrate, N-Glc-BE) is reported. The chemical and enzymatic stability of *N*-Glc-BE under different pH conditions and in human serum, along with lipophilicity and solubility, were evaluated. *N*-Glc-BE demonstrated chemical stability across various pH conditions but was hydrolyzed in the presence of esterase with a half-life of 8 minutes in human serum. The prodrug exhibited optimal solubility and a favorable hydrophilic-lipophilic balance. Its ability to permeate through skin layers was assessed using the Franz diffusion cell methodology. *N*-Glc-BE predominantly accumulated in the epidermis and dermis, and it reached the receptor compartment slowly and sustained over time while releasing its parent drug, BA. Finally, its biocompatibility was demonstrated in preclinical human skin models, including primary cultures.
In light of the results obtained, the new odorless prodrug *N*-Glc-BE proves to be a promising candidate for the transdermal delivery of BA, a molecule with multiple biological properties but a disadvantageous physicochemical profile. Using *N*-Glc as a carrier not only ensures non-toxicity but also leverages its important role in skin health and beyond.

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Novel Opportunities to Fight Pancreatic and Prostate Cancer Exploiting 1,2,4-Oxadiazole-Bearing Compounds

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Keywords: anticancer agents, pancreatic cancer, prostate cancer, covalent inhibitors, 1,2,4-oxadiazoles

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies, with an average 5-year survival rate of 11%, mainly due to the lack of early diagnosis and limited response to treatments. Prostate cancer is the second most frequent cause of cancer death in men, with around 1.2 million of new cases diagnosed each year. Chemoresistance is a key impediment for treating both diseases, and drugs with innovative mechanisms of action could allow to overcome resistance to therapies. The 1,2,4-oxadiazole ring is considered a privileged scaffold in drug discovery, and it constitutes the pharmacophore moiety of several anticancer agents. Starting from an in-house library of 1,2,4-oxadiazole derivatives, we evaluated the antiproliferative activity towards two different human prostate cancer cell lines, DU-145 and LNCaP. In addition, we investigated the ability of the two most active compounds to inhibit cell migration using wound healing assays in DU-145. These molecules were also tested in two different pancreatic cancer cell lines (PANC-1 and MIA PaCa-2) and they showed low micromolar IC50s. Early ADME-Tox investigation were performed to exclude drug-drug interaction, cardiotoxicity and cytotoxicity. In addition to the 1,2,4-oxadiazole ring, our molecules contain a 3-Br-4,5-dihydroisoxazole (BDHI)[1] nucleus, which is known to behave as a moderately reactive warhead. Thus, these compounds are expected to act as covalent inhibitors. Target(s) identification via mass spectrometry under denaturing conditions is currently ongoing, using PDAC cell lysates exposed to compound 16 under conditions mirroring those inducing antiproliferative effects. Overall, we identified novel agents for the treatment of pancreatic and prostate cancers, and their novel mode of action is currently being elucidated.

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Acknowledgements

This work was supported by the Guido Berlucchi Foundation Mini Grant CANCERCOPE, number G43C23002440001 (to C.B.).

Design and synthesis of novel FGFR inhibitors for the treatment of glioblastoma

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Keywords: Glioblastoma, FGFRs, Ponatinib, FGFRs inhibitors

Glioblastoma (GBM) is the most malignant type of glioma (grade IV) and is the commonest in adults, it accounts for more than 60% of all adult brain tumors. Surgery and radiation therapy were the traditional treatments for GBM. A substantial increase in overall survival was observed in 2005 with the addition of Temozolomide, an oral alkylating chemotherapeutic drug. Despite this improvement, GBM is still notoriously difficult to treat, has a high recurrence rate, and is linked to treatment resistance. [1] Therefore, it is urgent to find new therapeutic strategies to improve the prognosis of GBM. Fibroblast growth factor receptors (FGFRs) are a family of receptor tyrosine kinases (TKR) that comprises four highly conserved receptors homologs named FGFR1-4. FGFRs regulates various vital physiological processes such as proliferation, survival, differentiation and cell migration. The classical FGF/FGFR downstream signalling pathways include **Ras/Raf-MEK-MAPKs** (mitogen-activated protein kinases), phosphatidylinositol-3 kinase/protein kinase B (**PI3K/AKT**), **PLCγ**, and signal transducer and activator of transcription (**STAT**), depending on the cellular content in various cells and tissues. [2] The aberrant expression of FGFR1 and FGFR4, detected in GBM, is considered an oncogenic signalling pathway and is negatively correlated with overall survival. [3,4] In this context, we evaluated the molecules already known as FGFR inhibitors and inspired by Ponatinib chemical structure, a series of new molecules that may potentially inhibit the FGFR isoforms involved in GBM, were designed. Ponatinib is a multi-TK inhibitor which targets FGFR, SRC, ABL, PDGFR, VEGFR and is mostly crystallized with FGFR4 and FGFR1. For these reasons, we analyzed Ponatinib interactions in its binding pocket of FGFR1, assessed the chemical space in the target and conceived a variety of structural modifications on main scaffold to produce the Ponatinib-like derivatives: PL1-12. Synthesized PL compounds are evaluated on glioblastoma cell lines to determine the CC_{50} values while the kinase inhibition will be measured in vitro to calculate the IC_{50} values. Also selectivity amongst isoforms and other kinases will be assessed in vitro.

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AMYC BIOMED 2024

Novel PET hydrolases derived from thermophilic microorganisms

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Keywords: Plastics, biodegradation, PET hydrolases, health hazard

Millions of tons of plastic waste are generated every year due to the nature of their constituting polymers, which do not biodegrade or absorb into the environment. Consequently, the increase in plastic waste has become a pressing environmental issue.[1] Moreover, during their use, plastics may break down forming small fragments or particles with a diameter less than 5 mm, named microplastics.[2] These latter constitute an emerging environmental and human health concern. Indeed they have been found everywhere on earth, from the bottom of the oceans to polar regions.[3,4] Toxicological studies reported that the exposure to microplastics induces severe toxic effects, involving oxidative stress, metabolic disorder, immune response, as well as neurotoxicity. [5]
In recent years, biodegradation has emerged as a new green technology for upcycling plastic waste.[6] This process relies on the action of enzymes produced by microorganisms, which are able to break down plastic materials into their basic components, making them easy to recycle. In 2016, it has been reported that a bacterial strain called *Ideonella sakaiensis* 201-F6 was identified exhibiting the ability to produce two enzymes, polyethylene terephthalate hydrolase (PETase) and mono (2-hydroxyethyl) terephthalic acid hydrolase (MHETase). These enzymes enable *Ideonella sakaiensis* to use PET as their sole carbon source.[7,8] Since then, extensive research has been carried out to identify other bacterial strains capable of producing enzymes catalyzing plastics degradation.[1]
In this context, the identification of new bacterial hydrolases deriving from thermophilic microorganisms can give new insight into plastic biodegradation in harsh conditions, such as high temperatures. Through meta-genomic approaches, a novel thermophilic PET hydrolase, denominated PP PETase (PP), has been recently identified from geothermal samples. This novel enzyme was produced in recombinant form and purified to carry on it physico-chemical analyses and assess the thermal stability and structural features of PP in order to provide deeper insights into its functional and structural properties and identify its potential application.

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Oxysterols profile as marker of development stage in Zebrafish embryos

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Keywords: Oxysterols, Liquid Chromatography, Tandem Mass Spectrometry, Metabolism, Toxicology.

The investigation on oxysterols profile in a suitable in vivo system could provide useful information on the amount, roles and biological functions of these molecules that are enzymatically generated by cytochrome P450 (CYP450) family, or via autooxidation, or even via both pathways in synergy. Oxysterols, which are oxidized derivatives of cholesterol, are reliable indicators of oxidative stress and lipid oxidation.
Zebrafish (*Danio rerio*), due to their genetic similarity to humans and transparent embryos, serve as an excellent model for such studies. Zebrafish is used in toxicology to estimate the effects of xenobiotics and their teratogenic consequences; this animal model presents several advantageous features as high fecundity, rapid embryonic development (24 h) and external fertilization[1]. The knowledge of the oxysterols profile in zebrafish, during early embryonic stages, provides important information on the role and biological function of these molecules. Moreover, different oxysterols have been investigated in zebrafish as liver X receptor (LXR) activators of the liver and regulators in the metabolism of carbohydrates and lipids and the cytotoxic effects of 25-hydroxycholesterol on nervous system cells in zebrafish larvae was demonstrated[2].
This research focuses on the determination of oxidative stress in zebrafish embryos by assessing oxysterols as biomarkers at different developmental stages: 3, 24, 48, 72, and 96 hours post fertilization (hpf). The study employs liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the precise quantification and analysis of oxysterols in zebrafish embryos. The methodology involves the rapid extraction of lipids using a modified solid-phase extraction (SPE) technique based on the method described by Fanti et al. [3] in their study on oxysterol profiling in zebrafish embryos. Following extraction, separation and detection are conducted using LC-MS/MS, which offers high sensitivity and specificity. The method was validated according to European Medicines Agency (EMA) guidelines, ensuring robustness and reliability. The results demonstrate that specific oxysterols are significantly elevated in embryos exposed to oxidative stress, validating their use as biomarkers. This approach not only enhances our understanding of oxidative stress in aquatic models but also provides a robust tool for environmental and pharmacological studies.

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A Multidisciplinary Approach to Design and Characterize Essential Oil-based Nanoemulsions with Antibacterial Activity

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Keywords: Nanoemulsion, Antimicrobial, Drug Delivery, Nanomedicine

In recent years, antibiotic overuse has resulted in increase in the number of resistant bacterial pathogen strains. Essential oils (EOs), which already have antimicrobial properties, can be formulated into nanoemulsions (NEs) to enhance their properties and their efficiency against biofilms and multi-drug resistant bacteria. NEs, because of their hydrophobicity, can partition into lipids of bacteria cell membrane, disrupting its structure and making it more permeable, leading to the leakage of molecules and finally to cell death [1]. In this way, the carrier itself could be active and subsequently enhance the activity of the active loaded molecules.

The aim of the present study is to prepare and characterize oil-in-water (O/W) EOs based-NEs for the delivery of active molecules with antibacterial activity.

Among EOs, NEs have been formulated using a mixture of essential oils: *Rosmarinus officinalis* essential oil (REO) and *Thymus vulgaris* essential oil (TEO). It is known that *R. officinalis* can enhance the antimicrobial activity of Ciprofloxacin against *K. pneumoniae* at specific concentrations [2], while TEO has a strong ability to inhibit and eradicate biofilm, reducing the concentration of antibiotic conventionally used [3].

First, pseudoternary phase diagram was constructed to determine the appropriate ratio of surfactant, oil, and aqueous phase to obtain homogeneous O/W NEs (Figure 1). Various formulations have been then tested for their cytotoxicity and activity. To evaluate the cytotoxic effect *in vitro*, a lung cell line was treated for 24 hours with different concentrations of NEs to identify the one capable of not causing cytotoxic effects, using the MTT colorimetric assay. The first sample prepared showed a powerful antimicrobial action against Gram-negative strains but at concentrations that turned out to be cytotoxic. The preparation was therefore optimised, to obtain activity but not toxicity. The selected formulation (Figure 1) was analyzed in terms of hydrodynamic diameter, ζ-potential and polydispersity index by using a Malvern ZetaSizerNano 90S. Stability studies have been carried out at 25°C and at 4°C for 3 months and oil drop features were investigated in terms of microviscosity, polarity and fluidity by using two fluorescent hydrophobic probes: pyrene and 1,6-diphenyl-1,3,5-hexatriene.

Furthermore, assuming a pulmonary administration route, NEs integrity during the nebulization process was evaluated. With the aim to investigate NEs release capability in different media, Nile Red was employed. Additionally, MIC against *E. coli* ATCC 25922 and *K. pneumoniae* #17 in Mueller Hinton Broth (MH) and MH II Broth were tested.

The preliminary characterization has confirmed that REO and TEO-based NEs could be an efficient non-cytotoxic tool at the tested concentration with a mild antimicrobial activity. The next step will be the inclusion of antimicrobial peptides to evaluate the possible synergy of the formulation and the carrier itself.

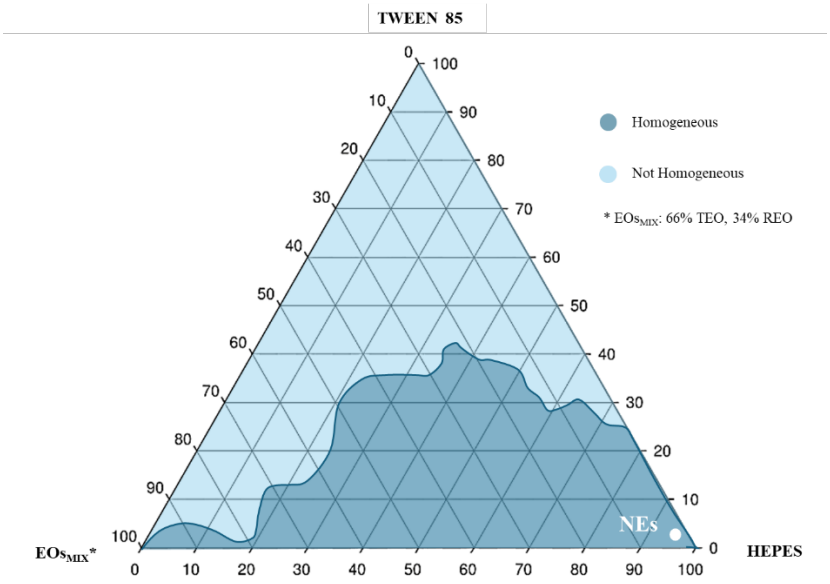

Figure 1. Pseudoternary phase diagram constructed to obtain homogeneous O/W NEs and the NEs selected

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Resolution of inflammation is impaired in primary human monocytes under microgravity

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Keywords: microgravity; resolution of inflammation; monocytes; specialized pro-resolving mediators; resolvins

Since the first Apollo mission in 1969 and the Skylab mission in 1973, microgravity has been considered a main space-related stressor responsible for cellular and molecular alterations of immune and inflammatory homeostasis, linked to the disorders that astronauts suffer during their missions and afterwards[1,2]. These disorders include bone and muscle mass reduction[3], neurological alterations, enhanced sensitivity to pathogens[4] and, overall altered immune and inflammatory homeostasis [5, 6]. Most of research of the past has established that adaptative immune cells represent a target of microgravity; among these monocytes and lymphocytes are important immune effectors. Of note, monocytes/macrophages are main producers of specialized pro-resolving mediators (SPMs), a novel class of ω-3- and ω-3-derived immunomodulatory lipids, synthesized through the action of 5-, 12- and 15- lipoxygenase (5-, 12- and 15-LOX) that include more than 45 bioactive compounds grouped in 5 main classes
classes, namely D- and E-series resolvins (RvD and RvE), maresins (MaRs), protectins and lipoxins (LXs).
These molecules are the main effectors of "resolution of inflammation" – i.e. the process that keeps at bay chronic inflammation by avoiding deviant activation of the acute immune pathways – and promote the removal of dead
cells and tissue debris, as well as they hinder pro-inflammatory features of virtually any immune cell involved in the inflammatory event [7, 8]. However, to date, microgravity has never been investigated in the field of pro-resolving lipids. Our results show for the first time that 24h exposure to rotary cell culture system (RCCS)-simulated
microgravity rearranges SPM receptors and enzymes both at the gene and protein level, in human primary monocytes, but not in lymphocytes. In particular we show a significant gene and protein upregulation of two SPM receptors, GPR32 and GPR18, and a significant decrease in 5-LOX expression simultaneously with a slight increase in its activity. The reduction in 5-LOX expression results in a significantly-reduced biosynthesis of RvD1, a prominent SPM. Also, we show monocytes and lymphocytes display reduced levels of pro- and anti-inflammatory cytokines, following RCCS incubation. Lastly, we show a reduction monocytes' production of TNFα at Earth gravity, but not at simulated microgravity, after the treatment with RvD1. These findings strongly suggest that not only microgravity can impair the functioning of immune cells at the level of bioactive lipids directly involved in proper inflammation, but it does so in a cell-specific manner, possibly perturbing immune homeostasis with monocytes being primary targets. Accordingly, this study is an important starting point in lipid space biology to further understanding of the molecular pathogenesis of diseases associated with space travel-related stressors and to design preemptive therapies that potentiate the pro-resolving network.

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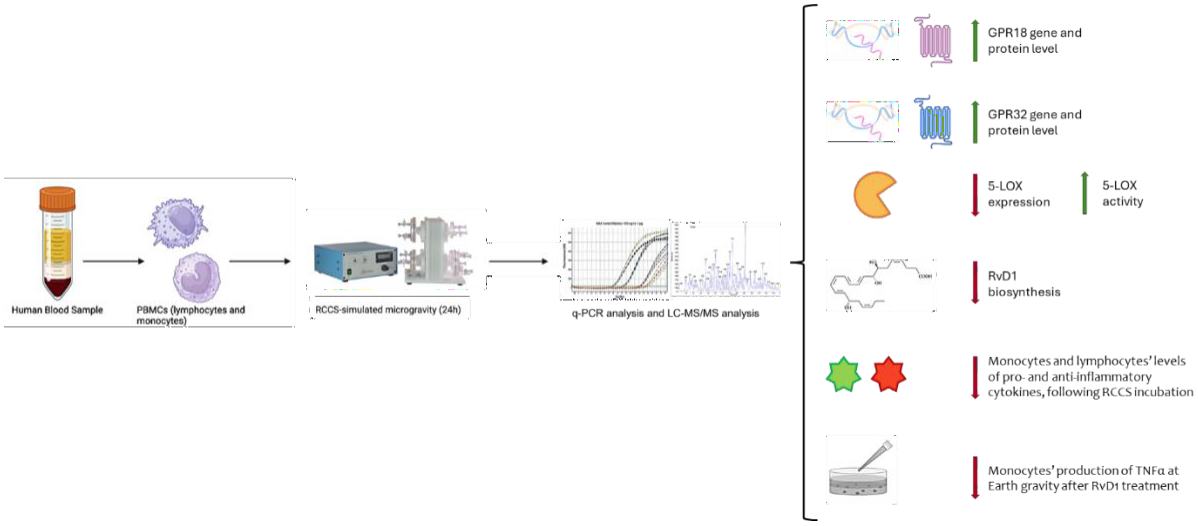
Figure:



The 24h exposure to RCCS-simulated microgravity upregulates, both at the gene and protein level in human monocytes, the SPM receptors GPR32 and GPR18; also decreases 5-LOX expression simultaneously with a slight increase in its activity. This results in a significantly reduced biosynthesis of RvD1. Further it reduces the levels of pro- and anti-inflammatory cytokines in monocytes and lymphocytes. Lastly, it reduces monocytes' production of TNFα at Earth gravity after the treatment with RvD1.

Acknowledgments: The Italian Space Agency (ASI) under the competitive project n. CE-DSR-UCO/2023-2, to

M.M. References:

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EFFECT OF CROCUS SATIVUS L. BY PRODUCT EXTRACTS IN RETINAL IN-VITRO MODELS OF NEURODEGENERATION

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Keywords: saffron, neurodegeneration, neurodegenerative diseases, retinal cells

Saffron treatment has been shown to be effective in slowing the progression of various neurodegenerative diseases, and its efficacy depends on a specific molecular composition (REPRON®)[1]. Its production requires specific cultures and procedures that, along with low yields, make this spice costly. To reduce costs, saffron from hydroponic cultivation is increasingly being used. It has already been demonstrated that hydroponic saffron and REPRON® have the same chemical composition and neuroprotective capacity following oxidative stress induction (H_2O_2)[2]. However, saffron production still requires high costs associated with the disposal of other parts of the plant that are not used. This study is therefore part of the circular economy context to evaluate whether extracts from other parts of this plant have the same protective properties as the stigmas. Retinal cell lines were pre-treated with extracts derived from different portions of plant waste. Subsequently, the cells were damaged using H_2O_2 500µM for 3 hours to induce oxidative stress, glucose 32mM for 48 hours for hyperglycemic model, and with lipopolysaccharide (LPS) 0,25 mg/mL to induce inflammation; the efficacy of the extracts was evaluated using biochemical techniques. REPRON® and hydroponic saffron extracts were used as positive controls.

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A NEW BIOTECHNOLOGICAL INDUSTRIAL PROCESS DEVELOPED FOR THE PRODUCTION OF HYALURONIC ACID IN BACILLUS MEGATERIUM

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Keywords: Hyaluronic Acid, Bacillus Megaterium, biotechnological fermentation, recombinant DNA technology, safe microorganism.

Hyaluronic Acid (HA) is a polysaccharide with a linear structure, naturally present in living organisms from bacteria to higher animals including humans. It belongs to the family of glycosaminoglycans (GAGs) and has a great capacity to bind water molecules forming a gel-like structure. This property derives from its structure: a long chain made up of repeated sequence of glucuronic acid and N-acetyl-glucosamine. Multiple biological roles of HA depend to a large degree on the size (molecular weight) of its chains despite molecular mechanisms of molecular weight control remain poorly understood not only for hyaluronan synthases but also for other beta-polysaccharide synthases, e.g. cellulose, chitin, and 1,3-betaglucan synthases. Since its discovery in 1934, HA has been used for its hydrating, repairing and regenerative properties and finally, for its ability to forming a sort of shock absorber and lubricating joints and other structures. HA is produced by extraction from tissues of animal and plant origin or through biofermentation and is currently used as active for viscosupplementation, wound care and cosmetics.
Fidia Farmaceutici S.p.A. is a company strongly committed to this sector because is a pioneer in the medical application of HA with the first approved HA-based preparation for the management of skin lesions: Connettivina. In the late 1980s, the company obtained marketing authorization for the first HA-based product for intra-articular administration: Hyalgan, a viscosupplement for the treatment of knee osteoarthritis.
In this work, we manipulated metabolite concentrations in the hyaluronan pathway by overexpressing the hasA (from *S. zooepidemicus*), tuaD, gtaB e pgi (from *Bacillus subtilis*) genes of the hyaluronan synthesis operon in bacillus megaterium by mean of recombinant DNA technology. Overexpression of these genes involved in HA biosynthesis led to a higher production of HA in *Bacillus megaterium* compared to the wild type strain which did not produce HA and in industrial fermenter increased the hyaluronan concentration from 1.6 to about than 3 gram liter compared to the pilote scale fermenter, achieving the high molecular weight to be observed at about 1200 kDa.
The data presented in this study represents the first industrial model of high-concentration hyaluronic acid in a safe microorganism (GRAS) capable of maintaining a high molecular weight based on the concentration of activated sugar precursors. These results can be used to engineer strains producing high molecular weight hyaluronan with high concentration and may provide insight into similar polymerization mechanisms in other polysaccharides.

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OMICS APPROACHES IN BIOREMEDIATION OF POTENTIALLY TOXIC ELEMENTS: INNOVATIVE SOLUTIONS FOR PROTECTING HUMAN HEALTH

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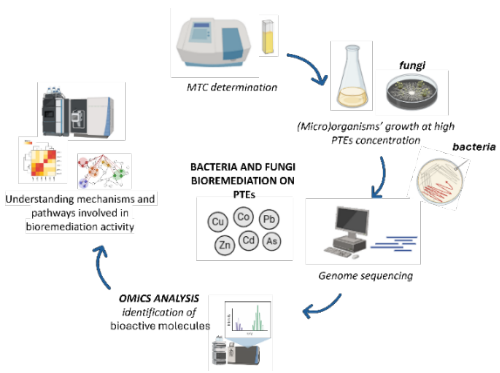
Keywords: bioremediation, PTEs, microorganisms, omics, human health

Uncontrolled population growth, intensive industrial production, and global industrialization have resulted in the alarming accumulation of xenobiotics and recalcitrant compounds, exerting a dramatic impact on both environmental integrity and human health. Among the different pollutants, heavy metals, more rigorously defined as potentially toxic elements (PTEs), are particularly concerning for their ability to accumulate in both terrestrial and aquatic environments and for their propensity to biomagnification and bioaccumulation. Both chronic and acute exposure to PTEs can lead to a wide range of adverse health effects causing from neurological disorders to cardiovascular disease and immunological system impairments. [1] [2] [3] [4]

To reduce PTEs accumulation and the subsequent damages to humans, a valid strategy can be bioremediation. This work, developed in collaboration with the *Stazione Zoologica Anton Dohrn* of Naples, aims to investigate the bioremediation potential of marine bacteria and fungi, isolated from polluted sites, to remove PTEs. The PTEs tested are As, Cd, Co, Cu, Zn, and Pb; these are selected based on their abundance in the Gulf of Naples and the Bagnoli area, site of the former *Italsider* steel mill, as well as considering their most toxic and prevalent oxidation states. The resistance of selected microorganisms towards the chosen metals is assessed, determining their maximum tolerance concentration (MTC). The metals are supplied in form of soluble salts at pH 7 to the microorganisms; metals concentrations in the range of parts per million (ppm) are chosen based on literature studies, to assess their toxicity. [5][6]

The most promising microbial strains are analyzed using complementary omics approaches to evaluate their bioremediation mechanisms and metal removal efficiency. Genomics identifies genes responsible for bioremediation activities, while proteomics and metabolomics elucidate mechanisms of PTEs biodegradation and bioaccumulation, identifying the bioactive molecules and the pathways involved and/or altered in the processes. This characterization aims at enhancing alternative routes of bioremediation with high-performance microorganisms, enabling the development of tailored strategies adapted to diverse environmental conditions and protecting human health. [5] 7][8][9][10][11]



Graphical abstract of the work

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Electrodeposition of silver nanoparticles on carbon electrodes to prepare electrochemical immunosensors

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Keywords: Electrochemistry, nanobiotechnology, immunosensor, silver nanoparticles

Electrochemical biosensors stand out as low-cost, portable and fast response time systems. Thanks to these advantages, they are promising systems for diagnosing diseases quickly and effectively. These systems are used in cancer diagnosis, infection detection and many biomedical applications [1]. Electrochemical biosensors enable non-invasive diagnosis of diseases from biological fluids such as blood, urine, saliva and pleural fluid [2], [3]. Thanks to these features, it becomes possible to detect the disease at an early stage and perform tests without patients feeling uncomfortable. Non-invasive diagnostic methods increase patient compliance as they cause less pain and discomfort, and also provide faster results. These capabilities of electrochemical biosensors have the potential to revolutionize the healthcare industry and play an important role in diagnostic processes [4].

In this study, an electrochemical immunosensor was developed for the determination of CRP in real samples. First, silver chloride/Mannose-4 aminophenylalanine (Ag/M4AF) was deposited on the surface using the electropolymerization technique to form silver nanoparticles. Then, covalent binding of Anti-CRP to AgNP/M4AF modified surfaces was carried out. AgNP/M4AF/Anti-CRP was characterized by electrochemical techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). Additionally, morphological characterization was performed with scanning electron microscopy (SEM). Finally, the linear range and limit of detection for electrochemical immunosensor were determined for CRP.

Acknowledgement
Ege University Scientific Research Projects Coordination Unit (Project Number: 25271) was acknowledged for financial support. S.S.Gelen thanks to TUBITAK 2211-A National PhD Scholarship Program.

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NMR Detection Of The Cellular Effects Of Gold-Based Anticancer Compounds

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Keywords: NMR, metal-based drugs, anticancer compounds.

^{1}H NMR provides a powerful tool to investigate the metabolic perturbations induced by gold-compounds [1] in cancer cells. The chemical identity and concentration of metabolites detected in cell lysates and their respective growing media by NMR can be viewed as a global fingerprint that unambiguously describes the response to drug treatment [2].
In this framework, we have carried out comparative NMR metabolomics studies to analyze the responses of A2780 human ovarian cancer cells to a panel of selected gold-compounds, including Auranofin, Aurothiomalate, Au(NHC)Cl and $[Au(NHC)_2]PF_6$. Due to the intrinsic nature of these metal centers, these molecules are supposed to give rise to multiple intracellular interactions with many functional proteins, rather than with a single enzyme or protein. Interestingly, with the proposed methodological approach, information on the predominantly affected biochemical pathways as well as on the protein targets of each metallodrug tested could be obtained and compared [3,4].

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Green synthesis of Prussian Blue Nanoparticles for multimodal therapy of breast cancer

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Keywords: Prussian Blue Nanoparticles, green synthesis, drug-delivery, breast cancer, photothermal therapy

Nanoparticle-based cancer therapy represents an emerging area of research dedicated to the development of more effective and less invasive treatments for oncological patients [1].
This study proposes an innovative synthesis of Prussian blue nanoparticles (PBNPs) for potential breast cancer therapeutic application. To improve the nanoparticles biocompatibility, the study focused on the green synthesis of PBNPs using natural stabilizing and reducing agents such as pectin, instead of traditional chemical reagents such as polyvinylpyrrolidone (PVP).
First, a comprehensive physicochemical characterization of PBNPs was performed. In addition, the ability of these nanoparticles to load and release Doxorubicin was evaluated. Then, the effect of both empty and doxorubicin-loaded nanoparticles on SKBR-3 breast cancer cells was investigated. Finally, the potential synergistic effect between the loaded nanoparticles and photothermal therapy was explored: the nanoparticles were irradiated with a laser to induce hyperthermia and thereby enhance the release of the chemotherapeutic agent. Preliminary results showed that laser irradiation improved the therapeutic efficacy of doxorubicin-loaded PBNPs, suggesting a promising combined approach for breast cancer therapy.
Overall, the data emphasize the potential of PBNPs as innovative therapeutic vectors that could significantly advance breast cancer treatments.

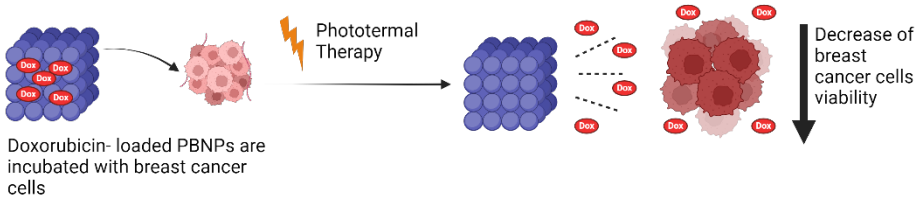

Figure 1. Schematic mechanism of Synergistic Action Between Doxorubicin-Loaded Nanoparticles and Photothermal Therapy.

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Discovery of a New Potent and Selective Histone Deacetylase 6 Inhibitor for Triple Negative Breast Cancer Treatment

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Keywords: epigenetics, HDAC6, breast cancer

Breast cancer (BC) is the most prevalent cancer spread among females worldwide. Hormone receptor-positive subtypes express both progesterone (PR) and estrogen receptors (ER), allowing BC growth and progression. Reliably, hormone receptor-positive BC can be efficiently treated with hormone therapy, with a positive impact on life expectancy. Unlikely, triple negative breast cancer (TNBC) accounts for about 20% of all BC subtypes, lacking the expression of PR, ER and HER2 receptors, thus rendering ineffective hormone therapy.[1] TNBC then represents the most aggressive BC subtype, accountable to its invasiveness and challenging therapy, with about one year life expectancy. TNBC cells show uncontrolled matrix remodeling leading to epithelial-mesenchymal transition (EMT), which can be related to uncontrolled cell invasiveness, proliferation, and differentiation. Recent studies pointed out the role of epigenetics in TNBC insurgence and progression, thus validating the hypothesis that targeting histone deacetylases (HDACs) could be an effective therapeutic option against TNBC tumorigenesis.[2,3] Herein we reported the discovery of a new spirocyclic compound (**1**) acting as potent and selective HDAC6 inhibitor (compound **1**, $IC_{50\ HDAC6}$ = 7.7 nM, $IC_{50\ HDAC1}$ = 2188 nM; Selectivity index HDAC1/HDAC6= 275), which was assessed for its efficacy in the MDA-MB-231 TNBC cell line. Compound **1** showed a highly efficient anti-proliferative effect in MTT assay, related to the activation of apoptotic process. Cytofluorometric assay also confirmed no effects on necrosis pathways, further validated by western blot analysis, with the detection of cleaved caspase-3, a reliable marker for apoptotic process. Also, increased levels of both Beclin-1 (BECN1) and Bcl-2 interacting protein 3 (BNIP1) were observed after 24 hours of treatment with compound **1**, thus also suggesting the involvement of the autophagic machinery in cell death induction upon selective HDAC6 inhibition. The *in vivo* validation of the of compound **1** is currently ongoing to confirm the promising *in vitro* outcome. Taken together, our results underscore the key role of epigenetic modifications driven by HDAC6 enzyme in TNBC tumorigenesis and cancer progression, providing a solid groundwork to reshape classical chemotherapeutic approaches.

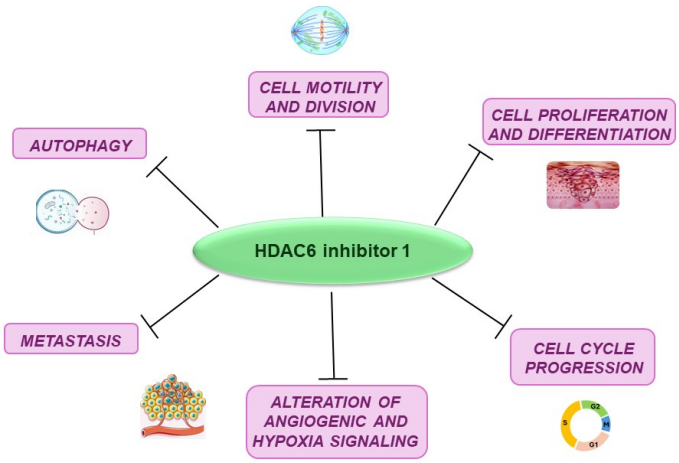

Figure 9- HDAC6 inhibitors in triple negative breast cancer.

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Electrochemical Immnosensor for the Determination of Serum Amyloid A (SAA) on Silica Nanoparticle Decorated Surfaces

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Keywords: Biosensor, electropolymerization, serum amyloid A (SAA), silica nanoparticle (SiNPs)

Immunosensors have great value for use in clinical area. These sensor systems are based on antigen-antibody reactions that are highly sensitive and specific. Moreover, electrochemical immunosensors are simple, fast, reliable, and inexpensive devices and can be used safely in clinical diagnosis [1]. The development of electrochemical immunosensor may enable miniaturization, and portability trends. It is also stated that small and low-cost nanomaterial-based electrochemical sensing devices will become more popular soon [2]. In this study, polydopamine (PDA) was synthesized primarily from dopamine (DA) by electropolymerization method. By modifying silica nanoparticle (SiNPs) surfaces with DA, PDA-SiNPs coated surfaces were obtained. Then, the serum amyloid A antibody (Anti-SAA) was covalently immobilized on the surfaces. All electrochemical characterizations in the experimental studies were performed by differential pulse voltammetry (DPV), cyclic voltammetry (CV), and impedance spectroscopy (EIS). To determine the effect of the number of cycles on the electropolymerization of DA-SiNPs, the number of cycles was optimized by applying deposition on the electrode surfaces for 10, 25, and 50 cycles. In the next step, covalent conjugation of Anti-SAA was performed by EDC/NHS chemistry. A calibration curve was created for SAA using the PDA-SiNPs/Anti-SAA biofunctional surface.

Acknowledgement

B. Karadag would like to thank TUBITAK-BIDEB for their support during her doctorate within the scope of the 2211-A Domestic Doctorate Scholarship Program.

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Surface functionalization of 3D-printed scaffolds with mucoadhesive properties

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Keywords: Mucoadhesion, bioadhesives, drug delivery, biopolymers, 3D-printing

In the regenerative medicine the scaffolds play a relevant role since they should deliver cells, drugs and genes into the body. They must have some qualities, such as the porosity and the biocompatibility, to induce tissue regeneration or to target a drug to a specific site [1]. 3D-printed scaffolds could confer personalized features that mimic the patient-specific architecture and the function of targeting tissues [2]. If the scaffolds are based on mucoadhesive polymers (natural or synthetic), they have the peculiarity of optimizing the localized drug delivery by keeping a drug in contact with the site of action for a certain period of time [3]. In particular, polymers can covalently/non-covalently interact with mucosal membranes thanks to their hydrophilicity and their functional organic groups [4]. Hydrophilic macromolecules, such as chitosan, sodium alginate and cellulose derivatives, are characterized by hydroxyl, carboxyl or amine groups, which can form hydrogen bonds and electrostatic interactions with mucin, a negatively charged, highly glycosylated protein, thus favoring the adhesion. Tablets, patches or microparticles are the forms under which traditional mucoadhesive materials can be commonly found and the drug is typically dispersed into them [5]. Chitosan (CS) is an ideal cationic polymer for this purpose thanks to its exposed amino and hydroxyl functional groups, its excellent biocompatibility, low toxicity and immune-stimulatory activities [6].In this work, an innovative type of biopolymeric based scaffold was proposed as a promising alternative to the traditional materials used to improve mucoadhesion. The scaffolds consisted in polylactic acid (PLA) structures, which were 3D-printed by Fused Deposition Modelling (FDM). Subsequently, they were submitted to alkaline hydrolysis. The CS coating was performed to obtain exposed hydroxyl and amino groups that can provide physical interactions with mucin. The printing parameters were optimized. The influence of the treatments on the thermal properties of the PLA structure was investigated by Differential Scanning Calorimetry (DSC). Scanning electron microscopy and contact angle measurements were performed to evaluate the efficacy of the CS deposition. In particular, the thermograms showed a sensible decrease in the intensity of the melting peak for the CS coated support in the second cycle. This suggested that the thermal properties of PLA were influenced by the presence of CS. The morphological analysis of the support showed that the alkaline treatment caused a highly porous surface, while the support surface after the CS coating was highly glossy, quite similarly to the neat untreated one. Hence, the PLA functionalization of the 3D-printed scaffold with CS successfully occurred. Contact angle measurements showed that the highest hydrophilicity of the surface was obtained when CS deposition occurred for one hour. In future, the mechanical properties and cytotoxicity will be investigated. Moreover, mucoadhesion tests will be performed to assess the adhesion behavior of the 3D-printed scaffolds.

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AMYC BIOMED 2024

Analyzing the impact of salt concentration on the DNA melting temperature

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Keywords: DNA, melting temperature, salt influence, thermocycler

As the fundamental building block of life, DNA has long been the focus of close scientific investigation. One interesting aspect of DNA is the impact of salt concentration on its melting temperature (T_m), a phenomenon that holds significant implications in various fields, including molecular biology, biotechnology, and genomics. The T_m of a DNA stretch is the temperature at which 50 % of the double-stranded DNA (dsDNA) molecules split apart into two single-stranded molecules (ssDNA), a process known as denaturation. The T_m is influenced by various factors, including the nucleotide composition of the stretch, the sequence length, and the ionic environment in which the DNA is dissolved. Recent studies have shed some light on the intricate relationships between the salt concentration and the DNA melting temperature[1–3]. Researchers have employed statistical models and numerical calculations to explore the stability of the dsDNA under a wide range of salt concentrations[1]. As the salt concentration increases, this stabilizes the DNA double helix, and the melting temperature of the DNA rises. In this context, the goal of the present work was to evaluate the impact of salt concentration on T_m through the fluorescent signal of SYBR Green I, which intercalates the dsDNA. To control the temperature of the process, this experiment was performed using a thermocycler applying a temperature sweep from 20 °C to 100 °C with a step increase of 0.5 °C. The samples were composed of 10 µM of each one of the 21 nt-long complementary ssDNA stretch, NaCl buffer with increasing concentrations from 0.1 mM to 1 M, and 50X SYBR Green I initially diluted in PBS and dH_2O. It is worth noting that the T_m trends when SYBR Green I was initially diluted in PBS and dH_2O were very similar, but the dilution of SYBR Green I in dH_2O induced a general decrease in T_m (*Figure 1*). This difference becomes more noticeable at low NaCl concentrations, suggesting that dsDNA is not as stable as at higher NaCl concentrations. Overall, the presence of PBS increases the stability of the dsDNA, and the T_m in all the conditions tested. According to the literature, the results confirm that as the salt concentration increases the stability of dsDNA is enhanced, which implies higher T_m.

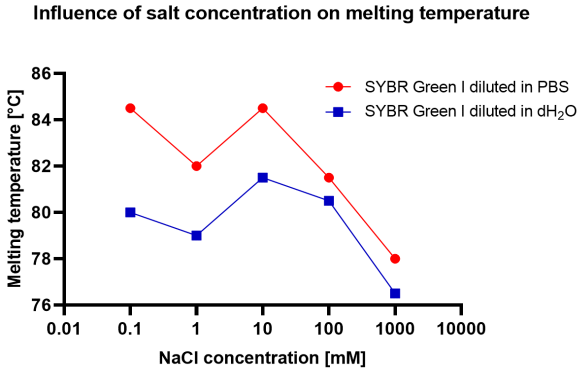

Figure 1. Dependence of Tm on the salt concentration. The red curve represents the samples prepared with SYBR Green I diluted in PBS, while the red one represents the samples with SYBR Green I diluted in dH_2O. For each dilution of SYBR Green I were tested at different NaCl concentrations of the buffers from 0.1 mM to 1 M.

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A novel NO-donor doxorubicin prodrug able to overcome multidrug resistance

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Keywords: Nitric oxide, Multidrug resistance, NO-donors, Doxorubicin, Cancer, Prodrug strategy.

Cancer remains one of the leading causes of death in developed countries, despite significant advances in understanding its mechanisms. Chemotherapy continues to be the preferred treatment method, but its efficacy is often hindered by multidrug resistance (MDR). This resistance is frequently mediated by transporters such as P-glycoprotein, which expel anticancer drugs from tumour cells. Efforts to inhibit these transporters with small molecules have proven challenging, prompting the exploration of alternative strategies.
Among various efflux pump inhibitors, nitric oxide (NO), a small gaseous molecule, has shown promise. However, NO cannot be administered directly; it must be generated by stable chemical entities known as NO donors (NODs) under specific conditions such as enzyme activity, subcellular localization, cellular factors, or light irradiation. Once produced in tumour cells, NO can initiate protein modifications (oxidation, nitrosation, nitration) that inactivate some of the transporters responsible for MDR, thereby increasing the intracellular accumulation of antineoplastic agents [1-2].
This approach's flexibility lies in the potential to chemically bind an antitumor drug with a NOD substructure. Furthermore, among the various known NO donors, those preferentially activated by enzymes or factors overexpressed in tumour cells can be selected to enhance selectivity.
Pursuing this line of research, we explored the possibility of conjugating doxorubicin (**DOXO**), an antitumor drug with a broad spectrum of action, with **FS506**, a prodrug of diazene diolate (also called **NONO-ate**) — into a single molecular hybrid known as **FS536**.
FS536 showed remarkable stability at physiological pH and in cell culture medium. After 24 hours of incubation in human serum, **FS536** demonstrated only 30% degradation, resulting in the release of 22% **DOXO** and 38% NO (quantified by the Griess assay). Biologically, **FS536** exhibited cytotoxic activity against the A549 lung cancer cell line, comparable to **DOXO**. In contrast, **FS506** alone was not toxic to the same cell line. In the **DOXO**-resistant A549-DR cell line, while **DOXO** showed no activity, **FS536** effectively inhibited cell proliferation, demonstrating its ability to overcome **DOXO** resistance and confirming the effectiveness of NO in this context. These findings highlight the potential of **FS536** as a promising strategy to combat MDR in cancer therapy, leveraging the synergistic effects of DOXO and NO for enhanced therapeutic efficacy.

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Real-time fluorescence tracking of the *in vitro* degradation of a vascular graft

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Keywords: medical device validation, degradation, fluorescence, vascular graft, in vitro

Introduction: To design a biodegradable medical device that is functional but, most of all, safe, an in-depth understanding of its degradation kinetic is needed. Several attempts have been made to obtain real-time monitoring of the device degradation through magnetic resonance imaging, X-ray computed tomography, and fluorescence imaging [1]. Among those, the fluorescence-based tracking method received the most attention since it can be used *in vitro* and *in vivo* without active contrast agents or complicated instrumentation [2]. After fluorescently labeling the material, its degradation can be tracked and quantified through fluorescence measurements. In our case, fluorescence analysis was coupled with protein quantification to track *in vitro* the degradation of the Silkothane® Arteriovenous Graft fabricated by Dialybrid that comprises the biodegradable and labeled silk fibroin (SF) and the non-degradable polyurethane [3].

Methods: 6 cm-long grafts were soaked into 0.1 M sodium carbonate-bicarbonate buffer (pH 9) with a fluorescein isothiocyanate (FITC) (Merck Life Science, Milan) concentration of 50 µg/mL, kept overnight at 4 °C. Then, the samples were washed twice (4 hrs and overnight) under shaking in MilliQ water. A labeled graft was incubated at 37 °C with Protease XIV (Merck Life Science, Milan) dissolved in 40 mL of PBS at a final concentration of 0.05 mg/mL. A stained control specimen was incubated with the buffer alone in the same conditions. Periodic sampling from both buffers was performed at 0.5, 1, 2, 4, and 8 hrs. Protein quantification using Bicinchoninic acid assay (BCA), and fluorescence measurements were performed. SF concentration was calculated by subtracting the enzyme contribution. Finally, the percentage cumulative release was determined for proteins and FITC [4]. The experiment was repeated twice.

Results: As expected, SF was released only by the digested graft during the time frame considered (Figure 1.a). Moreover, the amount of fluorescent probe released from the digested samples differed significantly from that eluted from the undigested one (Figure 1.b), meaning that a fluorescence signal can be related to the weight loss induced by enzymatic digestion. Finally, the cumulative release of SF and FITC showed similar trends, suggesting that tracking the fluorescence over time enables the prediction of graft degradation kinetics (Figure 1.c).

Conclusion: Through the methodology proposed hereabove, we could covalently and specifically label the SF in the graft and track the release of FITC-labeled SF induced by enzymatic digestion. These results allowed the validation of fluorescent SF labeling to characterize the graft's degradation kinetic *in vitro*. Moreover, although the protocol was optimized specifically for the Dialybrid product, the methodology developed can be conveniently adapted to other protein-based medical devices.

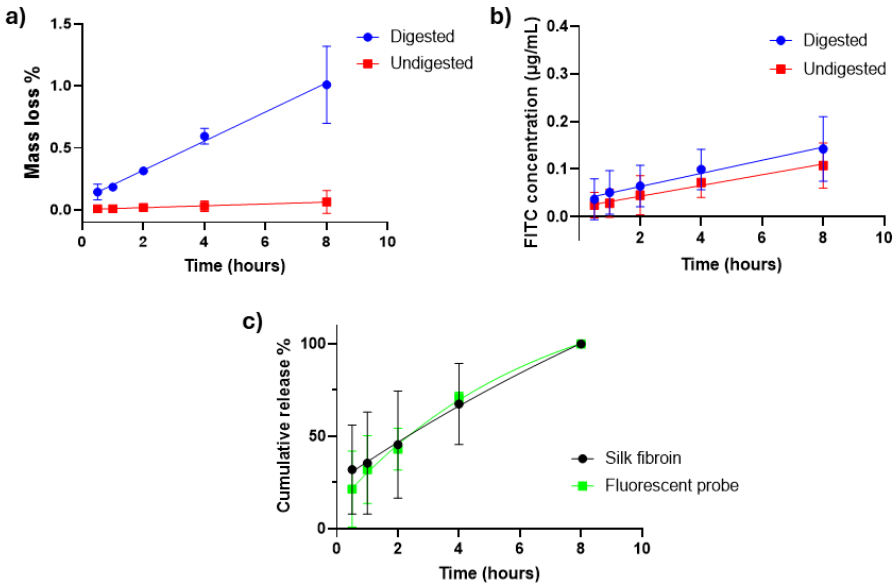

Figure 10: Graphs depicting a) the percentage mass loss and b) the measured FITC concentration in the buffers of digested and undigested samples over time. Graph c) shows the percentage of cumulative SF and FITC released from digested samples.

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Highly Specific N-Glycan Structure Monitoring using Signature Fragments

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Keywords: Critical Quality Attributes, Biotherapeutics, N-glycosylation, Tandem Mass Spectrometry

N-glycosylation is a critical quality attribute that affects safety, activity and pharmacokinetics of the biotherapeutics. The growing quantity of novel biological entities and process optimization necessitate the development of rapid workflows while maintaining sensitivity. The most comprehensive N-glycan profile characterization of glycoproteins will likely be provided by released N-glycan LC-MS/MS workflows; nevertheless, method development can be time-consuming and may call for a skilled operator. We have developed a rapid released N-Glycan workflow for monitoring several N-glycan structures. N-glycans have been enzymatically released in reducing conditions and labeled with fluorescent label RapiFluor-MS. Andrew+ pipetting robot has been used to reduce hands-on time and maximize traceability. Released N-glycans were analyzed with an Orbitrap mass spectrometer and XCalibur Software has been used for data analysis. One glycoprotein hormone expressed in Chinese hamster ovary (CHO) cells and one IgG-based drug expressed in SP2/0 murine myeloma cells together with two glycosylation variants of the latter have been used for this study. N-glycolylneuraminic, N-acetylneuraminic acids, α-galactose, core and arm fucoses and phosphated N-glycans are monitored structures. Signature fragments that are specific to sialic acids, core fucose and phosphated mannoses have been used to extract chromatograms from MS/MS signals. For α-galactosylation and arm fucoses relative ion intensities of signature fragments have been used to extract MS/MS ion chromatograms. Following exoglycosidases are used to demonstrate specificity of the signature fragments: sialidase A for N-glycolylneuraminic, N-acetylneuraminic acids; α1-3, 4, 6 galactosidase for α-galactosylation; α1-2 fucosidase and α3-4 fucosidase for arm fucose. After incubation with these enzymes, signals corresponding to target structures were disappeared. With this simplified qualitative workflow, N-glycan structures have been monitored successfully even at trace levels. Furthermore, we demonstrate the results are in accordance with orthogonal analysis previously performed by our group. Since the method is based on MS/MS fragments, presence of isomers or coelution does not present a challenge as is usually the case with FLR-LC-MS workflows.

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Multilayer of Prussian Blue Nanoparticles with photothermal antibacterial effect

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Keywords: Prussian Blue Nanoparticles, nanomaterials, antibacterial surfaces, photothermal effects

In this work, three self-assembled monolayers of PB (Prussian Blue) nanoparticles were grafted onto a functionalized glass substrate layer by layer in an attempt to create a photo-responsive antibacterial surface [1]. By using electrostatic interactions, cubic nanoparticles of colloidal PB were created and immobilized on layers of poly-L-lysine (PLL) and (PEI)-silane. The morphology and composition of the functionalized glass surfaces were extensively investigated through the use of UV-Vis spectroscopy, surface zeta potential measurements, SEM imaging, and photothermal effect study following 808 nm NIR laser light irradiation. The outcomes showed that the functionalized glass surfaces had consistent, even coverage, and that the number of PB nanoparticle layers rose linearly with the enhanced photothermal performance. The bacterial strain *Staphylococcus aureus* ATCC25923 (*S. aureus*) was used in biofilm condition to assess the antibacterial activity resulting from the photothermal effect of the surfaces. After being exposed to laser light for 30 minutes, the samples exhibited strong antibacterial effects that could reduce bacterial viability by as much as 30%. Results were further confirmed by confocal laser scanning microscopy (CLSM) observations. PBNP-functionalized surfaces represent promising candidates for future biomedical devices and implant applications.

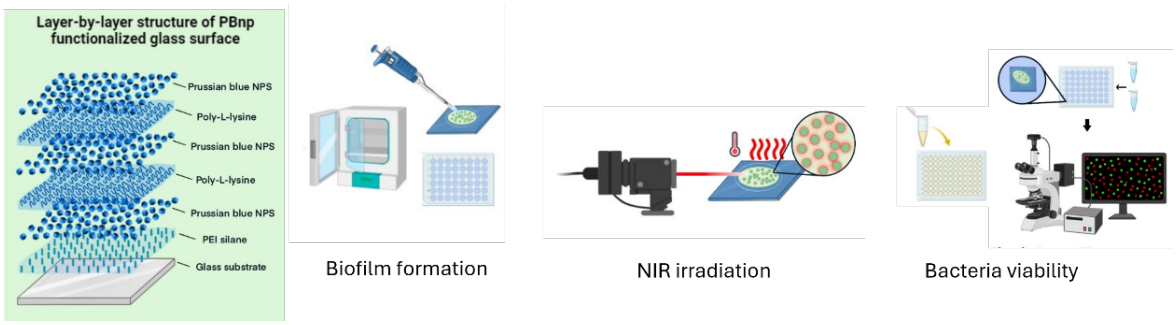

Figure 1. Schematic mechanism of the photothermal antibacterial effect of PBNPs functionalized glasses.

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Spatial multiomics in thyroid oncology: from phospholipids to glycopeptides analysis with 6-aza-2-thiothymine MALDI matrix

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Keywords: mass spectrometry imaging, 6-aza-2-thiothymine, multimodal, omics analysis, thyroid

Multiomics analysis employing spatial mass spectrometry imaging (MSI) presents a robust methodology for an in-depth molecular examination of biological specimens in order to investigate novel diagnostic, prognostic and predictive biomarkers. This technique enables the simultaneous mapping of multiple molecular layers, including proteins, lipids, and N-glycans, while maintaining their spatial information within tissues [1]. By integrating various molecular data from a single specimen, this approach minimizes the need for multiple tissue sections, which is particularly advantageous when dealing with limited material or precious samples. While 6-aza-2-thiothymine (ATT) has a historical use as a matrix for oligonucleotides and small molecules in matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) analysis, its potential in mass spectrometry imaging (MSI) has been relatively unexplored. [2]. In this study we first compared ATT performances with well-established MALDI matrices for both tryptic peptides (CHCA matrix) and lipids analysis (9-AA matrix). Subsequently, we present an integrated workflow for analyzing phospholipids, N-glycans through PNGase F enzymatic digestion, and trypsin-digested peptides from a single formalin-fixed paraffin-embedded (FFPE) tissue slice. Additionally, we proposed an innovative method for analyzing N-glycopeptides. ATT was used as the exclusive MALDI matrix for all targeted analytes. The ATT matrix was applied using an HTX TM-Sprayer, and MALDI-MS images were acquired using a rapifleX MALDI Tissuetyper mass spectrometer (Bruker Daltonics, Bremen, Germany). The versatility of ATT is notable for its minimal matrix clusters compared to other commonly used MALDI matrices, functioning effectively in both positive and negative polarity modes. ATT opens up a new scenario of analysis by enhancing the resolution and sensitivity of mass spectrometry imaging while preserving critical spatial information. This comprehensive approach allows for the detailed characterization of disease-related molecular changes, offering valuable insights into the molecular landscapes of various diseases.

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Diterpenoids from *Fabiana densa* var. *ramulosa* as colistin adjuvants in treatment of Gram-negative bacteria infections

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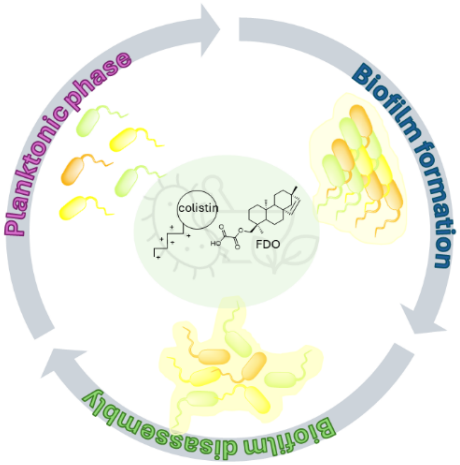
Keywords: diterpenes, antimicrobial activity, colistin, biofilm inhibition, extraction process

The emergence of multidrug-resistant (MDR) bacteria (those resistant to more than three antibiotic classes) has been paralleled by a waning antibiotic development pipeline [1]. The reintroduction in human medicine of last-resort drugs, such as colistin, has been followed by developing resistance mechanisms in Gram-negative bacteria. Studies have suggested a possible correlation between antimicrobial resistance and biofilm formation capacity [2]. Specifically, biofilms are composed of polysaccharides, proteins, and exogenous DNA [3] and can confer significant advantages to cells, helping in survival strategy and adaptation to a variety of stresses. Further, they play a critical role in the pathogenesis of chronic diseases such as tuberculosis and cystic fibrosis [4].
Recently, a tetracyclic *ent*-beyerene diterpene, *ent*-beyer-15-en-18-oxalate (FDO), has been identified and patented for its novel colistin adjuvant activity in Gram-negative cystic fibrosis pathogens [5]. In a previous study, this compound was isolated from aerial parts of *F. densa* var. *ramulosa* (Solanaceae), a native shrub of Chile, through solid-liquid extraction process and purification of the resinous extract performed by silica gel chromatography. Since a huge amount of FDO was needed in order to conduct a thorough biological characterization, a semisynthetic approach, based on the employment of the alcohol *ent*-beyer-15-en-18 -ol (FDA), already identified as chemical constituents of F. densa, has been developed [6]. Accordingly, the extraction procedure of FDA from the plant material has been lately optimized: the ultrasound-assisted extraction combined with the employment of an automated flash chromatography system allowed the isolation of FDA with a higher purity and increased yield (from 2% to 3,5%), reducing solvent waste and purification steps. FDA was then functionalized with oxalyl chloride affording the oxalate ester. Under these new experimental conditions, FDO has been obtained in large quantities and has been tested in biofilm inhibition assay. This compound, already known for its inhibitory activity on *P. aeruginosa* strain in presence of colistin, showed promising preliminary results as an anti-biofilm agent by preventing and controlling biofilm formation whenever when used in association with colistin.



Activity of FDO in association with colistin in different phases of bacterial life cycle.

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In vitro cytotoxicity of ibrutinib-loaded glycoconjugates based on mesoporous silica nanoparticles

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Keywords: Nanobiotechnology, glycoconjugate, nanoparticle, cancer

Breast cancer is the most commonly diagnosed cancer among women worldwide and remains a common health problem. Current breast cancer treatment includes surgery, radiation therapy, chemotherapy, and hormone therapy. To overcome the low specificity and serious side effects of conventional treatments, using nanomaterial-based drug delivery systems to maximize the lethal effect of chemotherapeutic agents on cancer cells and minimize undesirable side effects by providing better treatment targeting/localization are promising approaches.

Mesoporous silica nanoparticles (MSNs) with tunable particle size, large surface area and internal volume, uniform mesoporous structure, and multifunctional surface have been widely used as preferred nanocarriers over other drug carriers in recent years. The tunable pore diameter allows MSNs to transport drugs of various sizes for different cancer types. Moreover, encapsulation of drugs in MSNs can effectively prevent premature release and degradation, and at the same time reduce the toxic effect on healthy tissues.

Cancer cells typically express different glycans compared to normal cells, making carbohydrates attractive ligands for tumor-targeted drug delivery. Various carbohydrate groups, such as galactose, mannose, and glucose derivatives, are utilized as targeting ligands. The essential role of sugars may also be related to their association with lectins. Due to their strong association with lectins, carbohydrate-based drug development efforts have been intensively based on specific carbohydrate-lectin interactions.

Activation of the MSN surface with sugar ligands may maximize the therapeutic effect. In this study, MSNs coated with various sugar ligands were synthesized due to structural and type differences in lectin composition on cancer cells. The aim is to assess their toxicity and anticancer activity against cancerous cells based on the differences in sugar chemistry. Then, MSNs were coated with these sugar ligands. Anticancer drug ibrutinib was loaded into MSNs for drug release studies. Then, an *in vitro* study was performed on MCF-7 cell line. The obtained results suggest that sugar-coated MSNs are effective carrier platforms for controlled drug release.

Acknowledgement

D.S. Salmanoglu would like to thank The Council of Higher Education of Turkey 100/2000 PhD Project for their support (YOK 100/2000 PhD Project; special subject in Nanobiotechnology guided drugs)

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Enhancing Therapeutic Drug Monitoring with Aptamer- and Peptide-Based Biosensors

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Keywords: Therapeutic Drug Monitoring, Biosensor, Surface Plasmon Resonance

Therapeutic drug monitoring (TDM) is crucial to evaluate and improve treatment effectiveness, while enabling personalized treatments and precision medicine to enhance patient survival. Since drug effects vary among individuals, adjusting dosages based on individual pharmacokinetic profiles maximizes treatment benefits and minimizes adverse effects.
Until now, TDM is performed by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) analysis, which requires sample preparation and qualified personnel.
Our research activity focuses on aptamer- and peptide-based biosensing platforms that enable the recognition of small molecules with high-specificity and selectivity.
To this end, we aim at developing optical-based sensing platforms based on surface plasmon resonance (SPR). SPR biosensors are sensitive and provide results in real time, as they can track the kinetics of the recognition event. Furthermore, they are simple to use and highly automatable. In addition, they allow dynamic analysis of drug-protein interactions in real time, providing valuable information to optimize drug therapy.
We describe the use of SPR-imaging (iSPR) technique, which combines SPR technology with optical imaging, providing a detailed mapping of the distribution of molecules on the sensor surface. With this technology, we aim to revolutionize TDM by offering a rapid, accurate, and user-friendly alternative to traditional methods, ultimately enhancing personalized medicine and patient care.

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Unravelling the epigenetic mechanisms underlying the occupational carcinogenesis of hexavalent chromium

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Keywords: Human biomonitoring, cancer, workers, epigenetics, metal

INTRODUCTION
Approximately 900,000 workers in the European Union are exposed to carcinogenic hexavalent chromium (Cr(VI)). Genotoxic effects, such as oxidative stress and DNA lesions, have been recognized as crucial events in the carcinogenic process of Cr(VI) compounds. Moreover, it has been suggested that, in addition to the induced genotoxic effects, epigenetic mechanisms like DNA methylation may contribute to the carcinogenicity of Cr(VI) compounds.

AIM
Therefore, we investigated the epigenetic effects induced by occupational exposure to Cr(VI).

METHODS
We employed a cross-sectional study design and analyzed chromium in urine to characterize exposure to Cr(VI). Additionally, we investigated the effect of occupational Cr(VI) exposure on 8-hydroxy-2'-deoxyguanosine (8-OHdG), global DNA methylation and global DNA hydroxymethylation in blood. We included workers (n=254) from seven European countries with potential exposure to Cr(VI). As controls (n=114), we recruited healthy adult office workers from the same companies as the exposed workers (referred to as "within-company controls") or from other companies with no activities associated with Cr(VI) exposure (referred to as "outwith-company controls").

RESULTS
Overall, each exposed subgroup displayed significantly higher mean urinary Cr levels than the total controls, the within-company controls, or the outwith-company controls ($p < 0.007$, Mann-Whitney test). The within-company controls had significantly higher internal exposure levels than the outwith-company controls ($p < 0.001$, Mann-Whitney test). The outwith-company controls exhibited significantly higher global DNA methylation levels and lower levels of 8-OHdG than all other exposure subgroups ($p < 0.01$, Mann-Whitney test).

DISCUSSION/CONCLUSION
Overall, these findings reinforce the results of exposure biomarkers, highlighting that in Cr-related industries, (office) workers' exposure to Cr is associated with detectable alterations in biological effect markers. Furthermore, characterizing epigenetic effects like global DNA methylation could improve risk management.
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