Book of Abstract

AMYC-BIOMED 2025

6th Edition - Summer Edition

Palermo, Addaura Village Hotel, 23-25 June 2025

Organizing Committee

Giuseppe Domenico Arrabito (Local Chair), University of Palermo
Sara Anselmo, University of Palermo
Noemi Bellassai, University of Catania
Valeria Butera, University of Palermo
Michele Cacioppo, University of Palermo
Gabriele La Monica, University of Palermo
Annamaria Martorana, University of Palermo
Marina Massaro, University of Palermo
Silvia Orecchio, University of Palermo
Giorgia Puleo, University of Palermo
Giuseppe Sancataldo, University of Palermo
Dario Savoca, University of Palermo
Angelo Spinello, University of Palermo
Stefano Cinti (Chair), University of Naples Federico II

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Scientific Committee

Giuseppe Domenico Arrabito (Local Chair), University of Palermo
Sara Anselmo, University of Palermo
Noemi Bellassai, University of Catania
Alessandro Bertucci, University of Parma
Nina Bono, Polytechnic of Milan
Margherita Brindisi, University of Naples Federico II
Valeria Butera, University of Palermo
Michele Cacioppo, University of Palermo
Fabio Di Nardo, University of Torino
Concetta Di Natale, University of Naples Federico II
Nunzia Iaccarino, University of Naples Federico II
Gabriele La Monica, University of Palermo
Antonio Lupia, University of Cagliari
Annamaria Martorana, University of Palermo
Marina Massaro, University of Palermo
Marco Mendolicchio, Scuola Normale Superiore
Francesco Merlino, University of Naples Federico II
Morena Miciaccia, University of Bari
Rosalia Moreddu, University of Southampton
Silvia Orecchio, University of Palermo
Giorgia Puleo, University of Palermo
Deborah Quaglio, Sapienza University of Rome
Marianna Rossetti, ICN2 Barcelona
Giuseppe Sancataldo, University of Palermo
Dario Savoca, University of Palermo
Carla Serri, University of Sassari
Michele Schlich, University of Cagliari
Angelo Spinello, University of Palermo
Monica Terracciano, University of Naples Federico II
Stefano Cinti (Chair), University of Naples Federico II

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Program at a glance

Monday June 23rd		**Tuesday June 24th**		**Wednesday June 25th**	
08:00 **09:00** **09:40**	Registration Opening Wiley Workshop: **Dr. Francesca Rita Novara** *Opening Cerimony*	**09:00**	Session 4 – Mechanisms Chair **Marco Mendolicchio** *Oral Communications*	**09:00**	Session 8 – Green Chair **Dario Savoca** *Oral Communications*
10:00	Chair **Giuseppe Arrabito** Plenary Lecture, PL1: **Prof. Gianvito Vilè**	**10:00** **11:00**	Session 4S – Cancer Chair **Gabriele La Monica** *Oral Slams* Chair **Stefano Cinti** Plenary Lecture, PL2: **Prof. Andrew deMello**	**10:00** **10:50**	Session 8S – Scaffolds Chair **Carla Serri** *Oral Slams* Chair **Margherita Brindisi** Plenary Lecture, PL3: **Prof. Stefania Butini**
10:50 **12:20**	Session 1 – Biomaterials Chair **Marianna Rossetti** *Oral Communications* Sponsor Talk Buchi, ST1	**11:50**	Session 5 – Applications Chair **Marina Massaro** *Oral Communications*	**11:40** **12:35**	Session 9 – Biomimetics Chair **Michele Cacioppo** *Oral Communications* Sponsor Talk Instron, ST3
12:35	Session 1S – Multimolecules Chair **Fabio Di Nardo** *Oral Slams*	**12:35**	Session 5S – Applications Chair **Morena Miciaccia** *Oral Slams*	**12:45**	Session 9S – Sustainable Chair **Angelo Spinello** *Oral Slams*
13:40	Welcome Lunch *Poster Session 1,* P1	**13:40**	Lunch + Group Picture *Poster Session 2,* P2	**13:30**	Lunch *Poster Session 3,* P3
14:30	Session 2 – Prediction Chair **Nunzia Iaccarino** *Oral Communications*	**14:50** **16:00**	Session 6 – Cancer Chair **Deborah Quaglio** *Oral Communications* Sponsor Talk Alfatest, ST2	**14:15** **14:45**	Chair **Antonio Lupia** Invited Talk, IT3: **Prof. Alessio Malfanti** Session 10 – Structure-Activity

					Oral Communications
15:15 **16:10** **16:40**	Session 2S – Nanomaterials Chair **Concetta Di Natale** *Oral Slams* Coffee Break Chair **Nina Bono** Invited Talk, IT1: **Prof. Rosalia Moreddu**	**16:15** **16:50** **17:20**	Session 6S – RNA–centered Chair **Sara Anselmo** *Oral Slams* Coffee Break Chair **Michele Schlich** Invited Talk, IT2: **Prof. Laura Fusco**	**15:30** **16:00**	Session 10S – Nucleic Acids Chair **Noemi Bellassai** *Oral Slams* Coffee Break
17:10	Session 3 – Visualization *Oral Communications*	**17:50**	Session 7 – Natural *Oral Communications*	**16:20**	Awards
17:50	Session 3S – Light Chair **Giuseppe Sancataldo** *Oral Slams*	**18:20**	Session 7S – Metal-based Chair **Valeria Butera** *Oral Slams*	**16:30**	Closing Remarks
18:20	Social Activity	**20:30**	Social Dinner + DJ Set		

Full Scientific Program

Monday, June 23rd

8:00 **Registrations**
9:00 **Workshop, Francesca Rita Novara** - Wiley "Adapting your paper to a rapidly changing publication landscape"
9:40 **Opening**
10:00 **Plenary Lecture, PL1**
Chairperson: Giuseppe Domenico Arrabito
Gianvito Vilè - Polytechnic of Milan
"Green-by-Design Synthesis of Small-Molecule APIs via Atomically Precise Catalyst Design"
10:50 **Session 1, Biomaterials**
Chairperson: Marianna Rossetti
O1 Andrea Magrì, University of Catania
"Amyloid-b oligomers rewire VDAC1 electrophysiology and affect mitochondrial respiration in Alzheimer's disease"
O2 Claudia Pigliacelli - Polytechnic of Milan
"Self-assembly of short peptides towards functional nanomaterials"
O3 Sergio Scirè - University of Palermo
"PCI, a smart electrospun nano fibrillar biomaterial with magnetic, photothermal, and non-adhesive features for biomedical use"
O4 Giulia Turrin - University of Ferrara
"Enhancing biocompatibility of polyimide through surface covalent binding of dexamethasone for neural implantable devices"
O5 Giorgia Giovannini - Mario Negri Institute
"Design of bio-responsive nanomaterials as controllable carriers and diagnostic tools"
O6 Adriana Carolina Torres Mansilla - Institute of Science, Technology and Sustainability for Ceramics
"Bioinspired nanocrystalline surfaces for bone infection treatment: exploring the osteoinductive and optimized properties of antibacterial nanostructures"
12:20 **Sponsor talk, ST1**
Alice Pedruzzi - Buchi
12:35 **Session 1s, Multimolecules**
Chairperson: Fabio Di Nardo
OS1 Lourdes Rivas - Barcelona Institute for Global Health

"An aptamer-based rapid diagnostic test for the simultaneous detection of malaria and dengue"
OS2 Silvia Orecchio - University of Palermo
"Mitigation of environmental toxicity in perovskite solar panels through perfluoroalkylated additives"
OS3 Cristian Reale - University of Messina
"A fast separation method for coumarin and furocoumarins quantification in perfumes by means of supercritical fluid chromatography"
OS4 Alessia Bono - University of Palermo
"Synthesis, in vitro exploration, and reactivity studies of 6-chloropyridin-2-yl benzoates as promising covalent fragments with antiviral and pro-apoptotic activities"
OS5 Elisabetta Rosa - University of Naples Federico II
" Stimuli-responsive hydrogels from low-complexity peptide domains"
OS6 Sara Amata - University of Palermo
"Synthesis and SAR of 1,2,4-oxadiazole derivates as potential antibacterial agents"
OS7 Miryam Perrucci - University of Teramo
"Newest approach for micro- extraction from complex matrix"
OS8 Federico Magrin - University of Padua
"Lead optimization of specific OPA1 inhibitors enhancing cytochrome c release and cell death"
OS9 Ignazio Fiduccia - University of Palermo
"Proteomic insights into LRBA deficiency: investigating oxadiazole-based readthrough therapy"
13:40 **Poster session 1, P1 + Lunch**
14:30 **Session 2, Prediction**
Chairs: Nunzia Iaccarino
O7 Cinzia Franchin - University of Padua
"Strategies and technological approaches to reveal proteins in traces from plasma samples"
O8 Lisa Rita Magnaghi - University of Pavia
"A statistical approach towards biofilms hydrophilicity characterization integrating ANOVA, DOE, and PCA"
O9 Adriana Coricello - University of Urbino
"An ABMD-based workflow for predicting relative residence times of ligands against systems of pharmacological interest"
15:15 **Session 2s, Nanomaterials**
Chairperson: Concetta Di Natale
OS10 Maria Ruggirello - University of Siena
"Development of Nanobody Drug Conjugates (NbDCs) using SARS-CoV-2 as a model for targeted antiviral therapy"
OS11 Rossella Infantino - University of Palermo

"Highly functionalized hybrid carbon nano-forms materials for photodegradation of pollutants from water"
OS12 Annalisa Morelli - Mario Negri Institute
"Lung disorders treatment: Investigating the impact of a nasal nanoformulation in a murine model of pulmonary fibrosis"
OS13 Sara Manin - University of Catania
"Nanocrystals technology to enhance limitations of isoliquiritigenin"
OS14 Roberta Cillari - University of Palermo
"N,S-doped carbon nanodots conjugated with indoximod for a dual-mode approach in breast cancer immunotherapy"
OS15 Mattia Danilo Langellotto - University of Sassari
"Media milling optimization for rapid extraction of nanovesicles from lemon"
OS16 Elide Zingale - University of Catania
"Self-nanoemulsifying drug delivery systems (SNEDDS) improve curcumin's impact on retinal protection"
OS17 Denise Di Lena - University of Parma
"Single-step monitoring of bioavailable therapeutic antibodies using a protein-DNA nanoswitch platform"
16:10 **Coffee break**
16:40 **Invited talk, IT1**
Chairperson: Nina Bono
Rosalia Moreddu - University of Southampton
"Decoding bioelectricity in living systems"
17:10 **Session 3, Visualization**
Chairperson: Nina Bono
O10 Lorenzo Arrico - University of Pisa
"New Eu(III)-based luminescent probes for time-gating fluorescence microscopy"
O11 Jerneja Kladnik - University of Ljubljana
"Next-generation FDDNP analogues for amyloid β visualization"
O12 Andrea Cesari - University of Pisa
"The role of NMR Spectroscopy for the investigation of nanocarriers in drug delivery applications"
17:50 **Session 3S, Light**
Chairperson: Giuseppe Sancataldo
OS18 Giuseppina Biscari - University of Palermo
"NIR-responsive antimicrobial hydrogels based on gellan gum and bis-mpa polyester dendrimers"
OS19 Erika Maria Ricci - University of Chieti
"Rapid determination of solar UV filters in human whole blood, plasma and urine using an FPSE-HPLC pda method"
OS20 Michele Catacchio - University of Bari
"Few-Molecule Detection via SPR Enabled by pH Conditioning"
18:20 **Social activity**, Guided Tour in Palermo (ca. 2 hours)

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Tuesday, June 24th

9:00 **Session 4, Mechanisms**
Chairperson: Marco Mendolicchio
O13 Angela Parise - CNR-IOM, SISSA
"Revealing the intricate regulatory mechanisms of small Rho-GTPases"
O14 Stefania Melini - University of Naples Federico II
"Effects of N-acylethanolamines on metabolic damage and cellular stress mechanisms driven by obesity"
O15 Alessia Distefano - University of Catania
"Exploring cellulose nanocrystals as potential nanocarriers for copper ion delivery"
O16 Gabriele Cianfoni - Sapienza University of Rome
"Design and synthesis of linear polyamines for the development of highly performing ferritin-conjugates"
10:00 **Session 4s, Cancer**
Chairperson: Gabriele La Monica
OS21 Chiara Pastura - University of Catania
"Integrating microfluidics and plasmonics for capturing and detecting alterations in genomic DNA from circulating tumor cells"
OS22 Juliana Manchado - University of Palermo
"The effect of metal ion coordination on the photodynamic activity of chlorins for Photodynamic Therapy"
OS23 Marzia Fois - University of Ljubljana
"Mitochondrial KV1.3 inhibitors: A New Frontier in Anticancer Therapy
OS24 Maria Pia Caputo - University of Bari
"Multivariate quality control and machine learning for early pancreatic cancer diagnosis using the SiMoT platform"
OS25 Davide Ricci - University of Palermo
"New horizons in TP53 nonsense mutations treatment: unveiling novel translational readthrough-inducing drugs for cancer therapy"
OS26 Alessia Marconi - University of Bologna
"Innovative chemophototherapy strategies for cancer"
OS27 Francesco Cancilla - University of Palermo
"Waterborne polyurethane-based electroresponsive platforms for controlled chemotherapy delivery"
OS28 Martina Piga - University of Ljubljana
"Fighting Cancer at the Proton Gate: New HV1 Proton Channel Inhibitors"
OS29 Michele Menditto - University of Palermo
"TRIDs therapy for restoring p53 expression on stop mutated cells"
11:00 **Plenary Lecture, PL2**
Chairperson: Stefano Cinti
Andrew deMello - ETH Zürich
"Microfluidics for blood-based diagnostics".
11:50 **Session 5, Applications**

Chairperson: Marina Massaro
O17 Carla Rizzo - University of Palermo
"Fluorinated oxadiazoles as potential scaffolds for biomaterials"
O18 Attilio Marino - Italian Institute of Technology
"Biodegradable organic smart nanoparticles for biomedical applications on earth and beyond"
O19 Neda Bagheri - University of Rome "Tor Vergata"
"Synthetic DNA transducers: enhancing CRISPR-Cas12a applications beyond nucleic acids"
12:35 **Session 5S, Applications**
Chairperson: Morena Miciaccia
OS30 Serena Vittorio - University of Milan
"Integrating ligand and structure-based approaches to predict UGT-mediated drug metabolism"
OS31 Antonella Miglione - Univerisity of Naples Federico II
"Electrochemical screen-printed sensor for tacrolimus detection in whole blood: a step toward decentralize therapeutic drug monitoring"
OS32 Paola Sofia Cardinale - University of Palermo
"Adipose-derived stem cells and nutrigenomics: a new frontier in obesity treatment"
OS33 Nicole Pia Navatti - Univerisity of Naples Federico II
"Targeting fat-to-heart crosstalk in obesity: preclinical evidence on the effects of N-acylethanolamines mixture"
OS347 Alberto Deplano - University of Cagliari
"Harnessing hSTING for enhanced antiviral response"
OS35 Simona Barone - Univerisity of Naples Federico II
"Discovery of the first-in-class pyrazole-oxazole based synergistic antifungal agent: synergy studies, in silico investigation, and in vivo validation"
OS36 Miriam Cavinato - University of Milan
"PROTACs: a new strategy to target hDASPO degradation for the modulation of endogenous D-aspartate in schizophrenia"
OS37 Marco Banzato - University of Padua
"Synthesis of enantiopure (R)- and (S)-methadone metabolites via a cyclic sulfamidate approach and pharmacological evaluation as novel NMDAR antagonists"
OS38 Sara Mocka - University of Catania
"Synthesis of new 5-fluorouracil and heme oxygenase 1 inhibitor mutual prodrugs with antiproliferative effects"
13:40 **Poster session 1, P1 + Lunch + Group photo**
14:50 **Session 6, Cancer**
Chairperson: Deborah Quaglio
O20 Domiziana Masci - Catcholic University of Sacred Heart
"Novel dual-targeting carbonic anhydrase inhibitors: a promising strategy for cancer therapy"
O21 Patrizia Sarogni - Italian Institute of Technology

"Evaluation of rationally designed Topoisomerase inhibitors and FLASH radiotherapy for the treatment of head and neck carcinoma"
O22 Arianna Bonizzi - University of Milan
"A high-sensitivity approach for detection of breast cancer-derived extracellular vesicles using single molecule array technology"
O23 Panagiota Kalligosfyri - University of Naples Federico II
"Fully 3D-printed electrochemical sensor for miRNA detection in liquid biopsy applications"
O24 Giuseppina Santonoceta - University of Catania
"pH-responsive metal-coordinated assemblies for methotrexate delivery in cancer therapy"
16:00 **Sponsor talk, ST2**
Fabio Perissinotto - Alfatest
16.15 **Session 6s, RNA-centered**
Chairperson: Sara Anselmo
OS39 Thea Serra - University of Turin
"A novel lateral flow biosensor for the early detection of ovarian cancer-related microRNAs"
OS40 Andrea Deiana - University of Sassari
"Design and synthesis of a new generation of pan-RNA-virus inhibitors"
OS41 Alessandra Glovi - University of Naples Federico II
"Circulating miRNA-21 monitoring in Triple Negative Breast Cancer: a step toward portable liquid biopsy"
OS42 Elisa Schiavon - University of Padua
"siRNA therapeutics and the hurdles of delivery: insights from ANANAS nanoparticles"
OS43 Giulia Yuri Moscatiello - Mario Negri Institute
"Interaction of mRNA-based COVID-19 vaccines with lung adenocarcinoma cells"
16:50 **Coffee Break**
17:20 **Invited talk, IT2**
Chairperson: Michele Schlich
Laura Fusco - University of Padua
"MXenes from immune interactions to imaging applications"
17:50 **Session 7, Natural**
Chairperson: TBD
O25 Matteo Perra - University of Cagliari
"Nutriosomes as antioxidant carriers: a comparative study of cannonau grape pomace extract in a parkinson's disease mouse model"
O26 Angela Maione - University of Naples Federico II
"Innovative probiotic-enriched fermented juices: development and functional evaluation"
18:20 **Session 7s, Metal-based**
Chairperson: Valeria Butera

OS44 Sara Benetti - University of Pisa
"Anticancer potential of diiron bis-cyclopentadienyl complexes
OS45 Giorgia Puleo - University of Palermo
"Shaping ZnO synthesis through design of experiments to enhance photocatalysis and antibacterial activity"
OS46 Riccardo Rozza - CNR-IOM, SISSA
"Zinc-aided mechanism of splicing covalent inhibitor"
OS47 Salvatore Muscarella - University of Palermo
"Rationalizing the structural basis of organic-platinum hybrid complexes binding towards quadruplex-duplex hybrids through all-atom simulations"
OS48 Matteo Mari - University of Modena and Reggio Emilia
"Hydrophilic NO3Py derivative for Cu(II) coordination in nuclear medicine"
OS49 Gloria Gatto - University of Verona
"Cuproptosis: a new frontier in targeting PDAC cells"
OS50 Marianna Imparato - University of Naples Federico II
"Effect of a postbiotic-enriched diet on the antioxidant capacity of mytilus galloprovincialis exposed to Cu2O"
20:30 **Social dinner + DJ Set**

Wednesday, June 25th

9:00 **Session 8, Green**
Chairperson: Dario Savoca
O27 Giulia Di Prima - University of Palermo
"Environmentally friendly strategies to manage oromucosal oxidative stress-related pathologies: from grape processing by-products to polyphenol-rich buccal films"
O28 Chiara Maccari - University of Parma
"Environmental pollution and asthma: exposomic approach"
O29 Andrea Citarella - University of Milan
"Green synthesis and biological evaluation of 1,2,3-triazoles"
O30 Chiara Martino - University of Palermo
"The different effects of two global warming conditions on two sea urchin species exposed to environmental pollutants"
10:00 **Session 8s, Scaffolds**
Chairperson: Carla Serri
OS51 Rosella Prestia - Polytechnic of Milan
"Rational design of 3D-printed microfluidic columns for integration with chromatographic systems"
OS52 Ignazio Sardo - University of Pisa
"Engineering a high-affinity Fn3 scaffold protein for selective targeting of mesothelin in oncology"
OS53 Angela Pagliaro - University of Cagliari
"Rational design and synthesis of hSTING selective agonists as broad-spectrum antiviral approach"
OS54 Antonietta De Falco - University of Naples Federico II

"From repositioning to drug development: design and synthesis of novel captopril-inspired mbl inhibitors"
OS55 Mariya Ryzhuk - Sapienza University of Rome
"Aromatic abietane diterpenoids as a promising scaffold for the development of Arnt-mediated colistin resistance inhibitors"
OS56 Francesco La Monica - University of Palermo
"Seeding primary fibroblasts and microvascular fragments in plla scaffolds for a regenerative medicine approach"
OS57 Antonella Torre - University of Cagliari
"Computationally driven design of novel hSTING agonists for enhanced antiviral response"
10:50 **Plenary Lecture, PL3**
Chairperson: Margherita Brindisi
Stefania Butini - University of Siena
" Inhibitors of the endocannabinoids' catabolic enzymes as potential therapeutics for epilepsy and neuroinflammatory conditions: from selective inhibitors to multitarget directed ligands "
11:40 **Session 9, Biomimetics**
Chairperson: Michele Cacioppo
O31 Fabio Pizzetti - Polytechnic of Milan
"Decorated nanogels for modulating glial cells in spinal cord injury"
O32 Jessica Brandi - University of Verona
"Evaluating astaxanthin-loaded nanoparticles as drug delivery systems for ocular diseases: an LC-MS/MS-based investigation"
O33 Laura Di Muzio - Sapienza University of Rome
"Gelatin-based double network cryogels: influence of gelatin properties on mechanical and biological performance in 3D cell culture"
O34 Valentina Testa - University of Turin
"Groundbreaking NanoMIP technology for adenosine detection and integration in antibody-free bioassays"
12:35 **Sponsor talk, ST3**
Mario Verducci - Instron
12:45 **Session 9s, Sustainable**
Chairperson: Angelo Spinello
OS58 Francesca Terracina - University of Palermo
"Spray-dried soy protein-based microparticles encapsulating Phycocyanin from Spirulina for inflammatory bowel disease treatment: In vivo efficacy evaluation"
OS59 Andrea Capucciati - University of Pavia
"PDA and DHN melanin: an eco-friendly material for removing emerging contaminants from water"
OS60 Francesca Commito - University of L'Aquila
"Encapsulation of saffron in liposomes: formulation, stability, and applications"
OS61 Federica Meringolo - University of Calabria

"Amination of cellulose supports for preparation of antimicrobial materials"
OS62 Asma Munir - University of Bologna
"Sustainable biorefining of rice waste: extraction and utilization of value-added components for biomedical applications"
OS63 Anna Emanuele - University of Florence
"MIP-based sensor for the determination of PFOA in wastewater samples"
13:30 **Poster session 3, P3 + Lunch**
14:15 **Invited talk, IT3**
Chairperson: Antonio Lupia
Alessio Malfanti - University of Padua
"Exploring hyaluronic acid-conjugates for glioblastoma local chemo-immunotherapy"
14:45 **Session 10, Structure-activity**
Chairperson: TBD
O35 Davide Moi - University of Cagliari
"Tuning the dual HDAC6/Hsp90 inhibition by purine-based hydroxamic acids"
O36 Raffaele Pastore - University of Molise
"Deficiency in NPC2 results in disruption of mitochondria-late endosome/lysosomes contact sites and endo-lysosomal lipid dys-homeostasis"
O37 Paola Marzullo - University of Palermo
"Structure-activity relationship study of quaternary ammonium salts: synthesis, characterization, and antibacterial evaluation"
15:30 **Session 10s, Nucleic acids**
Chairperson: Noemi Bellassai
OS64 Ankita Sinha - University of Rome Tor Vergata
"DNA repair monitoring through an electrochemical scaffold sensor"
OS65 Sara Volpes - University of Palermo
"Aphanizomenon flos-aquae (AFA), a new potential therapeutic microalgae against aberrant DNA methylation and DNA damage"
OS66 Marta Marchesini - Polytechnic of Milan
"Optimizing hybridization temperature in nucleic acid-based point-of-care diagnostics"
OS67 Serena Gargano - University of Palermo
"DNA hypomethylation compromises chromosomal stability and drives defects in cell nucleus structure and mechanics"
OS68 Salvatore Martino - University of Palermo
"DNA methylation loss leads to failure in chromosome segregation faithfulness
16:00 **Coffee break**
16:20 **Closing Remarks + Awards**
List of poster contributions
Poster Session 1, P1
1: Emanuele Vitale - University of Palermo

"Targeting Nonsense Mutations: NV848, NV914, and NV930 for REP1 protein restoration in Choroideremia"
2: Asya Zerbato - University of Padua
"Peptides functionalized metal nanoparticles for dynamic cell interaction"
3: Dario Savoca - University of Palermo
"Does environmental warming enhance the toxic effects of phthalates on marine life?"
4: Antonio Bova - University of Pavia
"A novel chemometric strategy for in-situ electrical conductivity prediction of tap water in a flow system"
5: Vincenzo Piscopo - University of Palermo
"Design of ZnO nanostructures for the electrochemical sensing of creatinine in aqueous solution"
6: Margherita Massironi - Mario Negri Institute
"The effect of the temperature on the interaction between SLNPs loading a mRNA and target cells"
7: Atieh Mousavi - University College Cork
"Enabling early detection of ovarian cancer using a novel electrochemical sensing platform"
8: Francesca Bertini - Sapienza University of Rome
"Evaluation of the biocidal activity of Origanum vulgare L. essential oil in nanoemulsion"
9: Alessia Lo Re - University of Palermo
"Nanocomposite in situ forming hyaluronic acid hydrogels as potential bone filler for the regeneration of irregular bone defects"
10: Haidi Shehi -University of Milan
"Noncompetitive α amylase and α glucosidase inhibitors for type 2 diabetes treatment"
11: Giulia Maria Titone - University of Palermo
"Targeting the FLT3 receptor in AML therapy with Pharmacophore-Based Virtual Screening: An in silico approach to discovering new potential inhibitors"

Poster session 2, P2
12: Martina Stocco - University of Padua
"Platforms for therapeutic RNA/DNA delivery: Fusogenic ANANAS-based system for targeted delivery of siRNA/miRNA"
13: Riccardo Varrica -University of Palermo
"Antisense oligonucleotides as a therapeutic strategy in rare genetic diseases: applications in hutchinson-gilford progeria syndrome and duchenne muscular dystrophy"
14: Mayla Metitiero - Institut de Ciència de Materials de Barcelona
"Electrolyte-gated organic transistors for electrically monitoring the aggregation of oligoglycine tectomers"

15: Valentina Preziosi - University of Naples Federico II
"Assessing biomarkers in biological fluids by combining microfluidics and organic electrochemical transistors"
16: Armando Gisonna - University of Naples Federico II
"Celecoxib activates a ROS-mediated mitochondrial apoptotic program in 2D and 3D melanoma cell cultures"
17: Flavia Borrelli -Polytechnic of Milan
"Molecular dynamics-guided design of self-assembling antimicrobial peptides"
18: Lucia Abbatiello - University of Sannio
"Rational design and synthesis of novel alkylamides based on natural leads from zanthoxylum spp. and acmella oleracea and evaluation of their biological activity"
19: Alessia Berbiglia - University of Messina
"Selection and characterization of human single-chain antibody fragments capable of recognizing therapeutic anti-sense oligonucleotides bearing phosphorothioate backbones"
20: Anita Salmaso - Mario Negri Institute
"Nanoparticles for pulmonary drug delivery: MALDI-TOF analysis of imatinib-loaded liposomes after intranasal administration in a murine model of pulmonary fibrosis"
21: Alessandra Lipani - University of Palermo
"Identification of new small molecules as F508del-CFTR correctors for the treatment of cystic fibrosis"
22: Maria Luisa Perina - University of Catania
"Albumin conformational changes due to oxidation processes: a systematic approach"

Poster session 3, P3
23: Stefania Carbone - University of Naples Federico II
"Pyro-electrohydrodynamic approach for a high-sensitive and efficient jet printing of biomolecules"
24: Despoina Maria Varouchaki - Sapienza University of Rome
"Ferritin-polyamine conjugates for targeted delivery of siRNA therapeutics"
25: Guglielmo Emanuele Franceschi - University of Pavia
"Development of a chemo-assisted optical device for serum albumin sensing"
26: Gabriella Iula -University of Naples Federico II
"Electrochemical sensor to evaluate cellular toxicity through the analysis of nitrite ions"
27: Raj Paul- University of Rome
"DNA condensates as a platform for biosensing and therapeutic delivery"
28: Marta Cabibbo - University of Palermo
"Addressing the need for pulmonary siRNA delivery with polyaspartamide-based polyplexes"
29: Francesco Cairone - Sapienza University of Rome
"Kaki as natural anti-biofilm agent: strategies to control biofilm-forming candida"

30: Leonardo Brunetti -University of Bari
"An exploration of FPR2 signaling bias by ureidopropanamide derivatives"
31: Valentina Frusca -Scuola Superiore Sant' Anna
"Selenium nano-architectures selectively inhibit cell viability in vitro in hpv-negative oral carcinoma"
32: Angelica Fusi - University of Bologna
"Luminescent nanomaterial-enhanced immunosensing for personalized prostate cancer biomarker analysis"
33: Jelle Verdonck - KU Leuven
"Unravelling the epigenetic mechanisms underlying the occupational carcinogenesis of hexavalent chromium"
34: Mariia Savchenko- King's College London
"Magnetite mineralization inside cross-linked protein crystals"

PLENARY

Green-by-Design Synthesis of Small-Molecule APIs via Atomically Precise Catalyst Design

Gianvito Vilè[1]

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The sustainable production of active pharmaceutical ingredients (APIs) demands innovative strategies that minimize waste, energy consumption, and environmental impact from the earliest stages of process development. In this work, we present a Green-by-Design approach to the synthesis of small-molecule APIs, leveraging the power of atomically precise catalysts to achieve unprecedented levels of efficiency, selectivity, and scalability. By tailoring the catalyst structure at the atomic level, we enable highly controlled activation and transformation of molecular substrates, reducing the need for excess reagents and minimizing byproduct formation. Our methodology integrates catalyst design with process intensification principles, including flow chemistry and continuous manufacturing, to further enhance reaction sustainability and reproducibility. Comprehensive life cycle assessments (LCAs) confirm significant improvements in green metrics compared to conventional synthetic routes. This study highlights the transformative potential of atomic precision in catalysis as a cornerstone for next-generation, sustainable pharmaceutical manufacturing.

Microfluidics for blood-based diagnostics

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The past three decades have seen considerable progress in the development of microscale systems for use in the chemical and biological sciences. Interest in microfluidic technologies has been driven by concomitant advances in the areas of genomics, proteomics, drug discovery, high-throughput screening and diagnostics, with a clearly defined need to perform rapid measurements on small sample volumes. At a basic level, microfluidic activities have been motivated by the fact that physical processes can be more easily controlled when instrumental dimensions are reduced to the micron scale. [1]

Initially, I will briefly describe how microfluidic systems can be exploited to perform a variety of complex analytical processes and why the marriage of such systems with a range of optical spectroscopies provides a direct route to high-throughput and high-information content experimentation. I will provide examples of how contemporary microfluidic systems can be used to perform relevant experiments, such as nanomaterial synthesis, enzymatic assays and DNA amplification. [2]

I will then change tack and discuss how simple microfluidic systems can become powerful tools in blood-based diagnostics. We have been interested in leveraging the capabilities of microfluidic systems to manipulate, process and order micron- sized (e.g. cells) and nm-sized (e.g. extracellular vesicles) objects in a controlled and high-throughput manner. To this end, we have recently developed chip-based imaging flow cytometers that leverage either inertial or viscoelastic forces to perform ultra-high-throughput imaging of cells. [3,4] Using such systems, we can analyze cellular populations at rates approaching 500,000 cells/s. More recent activities have focused on developing microfluidic platforms able to measure the mechanical properties of cells at rates of up to 100,000 cells per second. Here, fluid elasticity is used to focus and deform cells. We have used this platform for a range of studies, including cell phenotyping, cytoskeletal drug analysis and identification of malignant lymphocytes in peripheral blood samples. [5] Finally, I will present a simple, viscoelastic-based microfluidic platform for label-free isolation of sEVs from human blood. [6] We hope that these platforms will open up new opportunities in the assessment of blood disorders, mechanical phenotyping of rare cells and sensitive diagnostic applications.

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Inhibitors of the endocannabinoids' catabolic enzymes as potential therapeutics for epilepsy and neuroinflammatory conditions: from selective inhibitors to multitarget directed ligands

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Keywords: Neurodegenerative diseases**,** fatty acid amide hydrolase (FAAH), and monoacylglycerol lipase (MGL), multitarget directed ligands (MTDLs).

Endocannabinoids (ECs) like anandamide (AEA) and 2-arachidonoylglycerol (2-AG) regulate CB1 and CB2 receptors, and control various signaling pathways. The enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) are responsible for terminating the signaling of ECs and controlling their levels, making them significant therapeutic targets. Selective or dual inhibition of these enzymes could amplify the positive effects of CB receptor activation while avoiding undesired psychotropic effects.

Epilepsy, the second most common neurological disorder, presents challenges due to the narrow therapeutic window of many anti-epileptic drugs, which require close monitoring. Despite therapeutic advances, drug-resistant epilepsy remains a significant issue. In temporal lobe epilepsy (TLE), AEA levels are lower, and increasing these levels in the hippocampus, without affecting 2-AG, can protect against excitotoxicity. FAAH inhibitors, such as the potent and selective **NF1245** and its analogues [1, 2] have shown potential in treating epilepsy by improving acute epileptic symptoms and preventing oxidative damage in the hippocampus, without cytotoxic or cardiac side effects.

Neuroinflammation, driven by microglia and astrocytes, is a key factor in neurodegenerative diseases and conditions like multiple sclerosis. Chronic inflammation leads to neuronal death due to oxidative stress from increased ROS species caused by mitochondrial dysfunction. FAAH inhibitors embedding ionizable functions have been designed to improve water solubility and metabolic stability, could reduce ROS production in astrocytes and exhibited anti-inflammatory effects in hippocampal slice cultures. [3] Additionally, irreversible MGL inhibitors have been developed to enhance brain permeability, increasing the ECS's therapeutic potential.[4]

The development of multitarget directed ligands (MTDLs) that target multiple receptors is an emerging strategy in treating neurodegenerative diseases. This approach includes targeting both the histaminergic H3 receptor (H3R) and histone deacetylase 6 (HDAC6). The H3R regulates neurotransmitters' release and is a key target in treating CNS-related disorders. New small molecules, impacting ECs' metabolism and H3R activity, have been developed bya a pharmacophore merging approach.

HDAC6 is a metalloenzyme that plays a key role in neurodegenerative diseases. Inhibiting HDAC6 may have therapeutic benefits, and recent research has led to hybrid ligands that target both HDAC6 and either FAAH [5] or MGL. These dual-target inhibitors may provide more effective treatments by simultaneously addressing multiple pathways involved in neurodegeneration.

In conclusion, targeting the endocannabinoid system through FAAH and MGL inhibition combined with new strategies, including MTDLs, offers significant potential for treating conditions driven by neuroinflammation and oxidative stress.

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INVITED TALKS

Decoding Bioelectricity in Living Systems

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Keywords: systems biology; bioelectricity; cancer; biotechnology; artificial intelligence

Bioelectricity is emerging as a fundamental layer of biological regulation beyond cellular excitability, shaping processes from embryogenesis to cancer progression. My talk will introduce the central role of bioelectric signaling in living systems, emphasizing how ion flows and membrane potentials encode functional states across biological levels. I will present recent advances in recording electric signaling in cancer cells, including high-resolution electrophysiological techniques that reveal spatiotemporal patterns of activity linked to malignancy. In parallel, I will highlight nanotechnology-based tools for probing and perturbing bioelectricity in single cells. These insights define a small part of the understanding of cancer as a multiscale dynamic and adaptive system. In the final section, I will briefly connect this bioelectric layer to systems biology, presenting emerging frameworks relevant to artificial intelligence, and how bridging these seemingly diverse approaches might offer a powerful integrated tool to uncover cancer complexity.

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MXenes: from immune interactions to imaging applications

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Keywords: MXenes, 2D Nanomaterials, Immune System, Imaging, CyTOF

Thanks to their distinctive physicochemical properties, two-dimensional (2D) nanomaterials have garnered increasing attention across a wide range of fields, including nanotechnology, energy, and biomedicine. We recently introduced the "NanoImmunity-by-Design" concept, which expands the characterization of 2D materials beyond their physical and chemical features to include their immunological profiles. [1] Such immune profiling requires high-dimensional and information-rich approaches to fully capture the complexity of the immune response. [2,3]
To this end, we have employed advanced technologies such as single-cell mass cytometry (CyTOF) and imaging mass cytometry to investigate the immunological properties of graphene and emerging 2D materials like transition metal carbides/carbonitrides (MXenes). [4–6] We demonstrated that amino-functionalization of graphene oxide enhances its immunocompatibility [4], and showed that combining graphene with $AgInS_2$ nanocrystals enables its robust detection by CyTOF across a broad range of primary immune cell. [5]
More recently, we explored the immunomodulatory effects of selected MXenes and achieved their label-free detection using CyTOF and high-dimensional imaging through our LINKED approach. [6–7] We also extended the LINKED methodology to the study of environmental nanopollutants, including nanoplastics. [8]
Building on this work, we will present unpublished results on a broader array of novel 2D materials. [9–10]
Altogether, our findings establish that the chemical and immunological design of 2D nanomaterials can unlock new avenues for their safe and effective use in biomedicine.

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Exploring Hyaluronic Acid-Conjugates for Glioblastoma Local Chemo-Immunotherapy

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Keywords: Drug Delivery, Glioblastoma, Hyaluronic Acid, Local treatment, Immunotherapy

Glioblastoma (GBM) is the most prevalent primary brain cancer. The standard of care therapy (Stupp protocol, unchanged since 2005) includes maximal safe resection of the accessible tumor followed by radiotherapy and chemotherapy using temozolomide combined with corticosteroids. Despite the invasive treatment, the patient's median survival is 14.6 months. Traditional and immunotherapy-based treatments for GBM currently lack efficacy, remaining an unmet medical need. The difficulties faced by current approaches relate to: *i)* the blood-brain barrier (BBB) excluding most therapeutics (e.g., immune checkpoint inhibitors, ICIs); *ii)* the GBM stroma hampering the diffusion of therapeutics; *iii)* highly infiltrative cancer cells, *iv)* immunosuppressive Tumor Immune MicroEnvironment *v)* patient variability, and *vi)* standard-of-care destroying the immune system. Therefore, new therapeutic approaches are needed to improve the outcomes.

Polymer-drug conjugates have attracted significant attention for cancer therapy. The advantages of conjugating therapeutics to a polymer backbone reside in the ability of conjugation to provide a rationally designed linking chemistry that allows for sustained and site-specific drug release, thereby extending the half-life of drugs in clinical settings. Additionally, polymeric carriers can enhance the therapeutic performance of drugs by improving organ accumulation, promoting intracellular uptake, and reducing side effects.

Hyaluronic acid (HA) has the potential to improve the performance of the drugs alone, being an optimal platform to develop a polymer-based *in situ* chemoimmunotherapy against GBM. HA has been chosen due to its interaction with CD44, Toll-like (TLRs)-2 and 4 receptors, overexpressed in cancer and immune cells. Administration of HA-drug conjugates as a class of biodegradable nanomedicines for localized treatment represents a less explored area that may fulfill the potential of GBM. Overall, our results emphasize that HA-drug conjugates constitute an effective drug delivery platform for local chemoimmunotherapy against GBM. Our findings support the future exploration of HA as a versatile immunotherapeutic tool for improving clinical outcomes of other aggressive tumors in combination with various chemo-immunotherapeutics or cancer vaccines.

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ORAL COMMUNICATIONS

Amyloid-β oligomers rewire VDAC1 electrophysiology and affect mitochondrial respiration in Alzheimer's disease

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Keywords: Mitochondria, VDAC1, Alzheimer's disease, amyloid-β (Aβ) peptide, electrophysiology.

The Voltage-Dependent Anion-selective Channel 1 (VDAC1) is the most abundant pore-forming protein of the outer mitochondrial membrane (OMM). Being substantially a channel, VDAC1 allows the continuous exchange of ions (Cl^-, K^+, Ca^{2+}, Mg^{2+}), ATP/ADP, NAD^+/NADH and Krebs' cycle intermediates between the cytosol and mitochondria. In addition, VDAC1 interacts with cytosolic enzymes, such as Hexokinases (HKs), coupling the oxidative phosphorylation to glycolysis and, thus, playing a pivotal role in the regulation of the whole cellular bioenergetics. However, alterations in VDAC1 expression and/or activity have been observed in many pathological conditions, including Alzheimer's disease (AD), the most common neurodegenerative disorder in the elderly, and correlate with the impairment of specific mitochondrial functions. At the molecular level, AD is characterized by the intra- and extra-cellular accumulation of aggregates of amyloid-β (Aβ) peptide. Consisting mainly of 42 amino acids, Aβ peptides are generated from the aberrant cleavage of the Amyloid Precursor Protein (APP), an integral protein that principally enriches the plasma membranes of neuronal synapses. Being highly hydrophobic, Aβ peptide is particularly prone to self-assembly into multimeric forms, such as fibrils – the key component of extracellular amyloid plaques – and intermediate products of low molecular weight, oligomers and protofibrils. Particularly, Aβ oligomers are highly neurotoxic as they accumulate within the neurons, diffuse from one cell to another and aggregate onto the cytosolic surface of mitochondria, using VDAC1 as a main docking site.
In light of this evidence, here we investigated the effects of Aβ oligomers on VDAC1 functions, mainly through electrophysiological and respirometric approaches. Our findings indicate that Aβ oligomers significantly modify the physiological conductance, voltage-dependency and kinetic features of VDAC1, as well as its slight selectivity for anions, leading to a marked preference for cations, including Ca^{2+}. Given that VDAC1 is mainly involved in the trafficking of charged molecules in and out of mitochondria, a general reduction of cell viability and mitochondrial respiration was detected in permeabilized neuroblastoma cells and intact primary rat cortical neurons exposed to Aβ oligomers. Interestingly, the toxic effect mediated by Aβ oligomers was drastically counteracted by the use of a VDAC1-antagonist small synthetic peptide, NHK1, designed on the N-terminal domain of HK1, the most common physiological interactor of VDAC1. On these results, VDAC1 emerges as a crucial molecule in mitochondrial dysfunction in AD and as a promising pharmacological target for the development of new therapeutic avenues for this devastating neurodegenerative disease still without a cure.

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Self-assembly of short peptides towards functional nanomaterials

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Keywords: peptides, self-assembly, nanocarriers, functional additives, hybrid systems

Peptide self-assembly represents a versatile tool for creating nanoscale materials with tuned structures and functionalities. Despite the modular nature of peptides, their affirmation as building blocks in bio-nanomaterials design faces challenges such as high production costs for long sequences and susceptibility to proteases. Our work in this field is focused on the design of different self-assembling peptides able to form custom nanosystems and to act as functional additives and/or templates in hybrid biomaterial design. [1,2] At this aim, we explored the use of the short peptides to modulate the self-assembly and properties of biopolymer-based nanosystems, proving the ability of the selected peptides to act as functional additives. [3] More recently, we devised peptide nanovesicles obtained *via* the self-assembly of tri- and pentapeptides. By tuning the amino acidic sequence features, the nanovesicles stability in biorelevant fluids and their resistance to proteolytic degradation can be modulated. Notably, both hydrophobic and hydrophilic cargos could be hosted by the peptide nanovesicles, with the drug release triggered and controlled by proteases activity.

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PCI, a Smart Electrospun nano fibrillar biomaterial with magnetic, photothermal, and non-Adhesive features for biomedical use

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Keywords: Electrospun nanofiber, Near-infrared (NIR) photothermal activation, magnetic iron oxide nano-powder (Fe_2O_3), post-surgical adhesion, wound dressing, poly(butylene succinate).

This work was aimed at creating a biomaterial with applications in biomedicine, particularly in situations where non-adhesive surfaces are needed—such as preventing tissue adhesions after surgery. Surgeries involving the abdomen, gynecological areas, or joints often lead to pathological adhesions that interfere with organ function. A non-adhesive material can serve as a temporary barrier during healing, preventing unwanted tissue connections. [1] Similarly, implants like pacemakers and joint prostheses face the risk of fibrotic encapsulation, which diminishes their function. [2][3] Anti-adhesive coatings can reduce the body's fibrotic response, improving compatibility. [4] In wound care, a drug-releasing material that inhibits cell adhesion can provide protection without hindering natural skin regeneration. [5] To address these needs, we developed PCI, a nanofibrillar biomaterial made via electrospinning from a blend of poly(butylene succinate) (PBS), iron oxide nanopowder (INP, <50 nm) for magnetic responsiveness, and ciprofloxacin (CPX) for its therapeutic effect. INP enables MRI visibility and remote control with magnetic tools, and also generate heat when exposed to near-infrared (NIR) light.
Scanning electron microscopy (SEM) showed a fine fibrous structure (~0.4 µm in diameter), and energy-dispersive X-ray (EDX) analysis confirmed Fe on the surface. X-ray micro-computed tomography (µ-CT) revealed uniform distribution of INP in the material. The concentrations and release profiles of iron and CPX in phosphate-buffered saline (pH 7.5) were assessed and UV analysis, showed a CPX loading efficiency of 100 ± 5% and INP at 74 ± 3%, with minimal material loss during fabrication. No detectable iron release occurred under test conditions. CPX exhibited sustained antibiotic release, reaching 30% over 96 hours, making PCI suitable for use in wound dressings. [6] ATR-FTIR confirmed the integration of CPX through identifiable infrared peaks. Differential scanning calorimetry (DSC) of electrospun scaffold only showed PBS's melting peak, indicating that CPX became amorphous during fabrication, altering its thermal traits. Mechanical testing revealed a Young's modulus of 19.4 ± 1.9 MPa, maximum tensile stress of 2.9 ± 0.3 MPa, and maximum strain of 30.2 ± 3 %, which are suitable for handling in biomedical settings. [7] MTS assays on fibroblasts and keratinocytes confirmed that PCI is biocompatible yet non-adhesive to cells.
Drug release was further enhanced under NIR light, increasing CPX release to 45% over 96 hours, demonstrating modulation of controlled release through external stimulation. These results highlight PCI's promise as a smart drug-delivery platform, offering high local antibiotic concentrations, reduced systemic side effects, and better patient compliance. Additionally, PCI could offer therapeutic advantages in clinical and surgical scenarios where tissue adhesion or fibrosis is problematic.

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Enhancing Biocompatibility of Polyimide through Surface Covalent Binding of Dexamethasone for Neural Implantable Devices

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Keywords: dexamethasone, drug delivery systems, surface chemistry, foreign body reactions, intraneural electrodes.

Neural implants are widely used in prosthetic applications to interact with the peripheral nervous system, but their long-term functionality is compromised by the foreign body reactions (FBR), an inevitable process occurring whenever intraneural devices are implanted: the excessive inflammatory response leads to the growth of fibrotic tissue around the inserted device, isolating it from the target already two weeks after the surgical implantation. [1] Polyimide *poly*(biphenyl dianhydride)-*p*-phenylenediamine (BPDA-PDA) represents a suitable material to fabricate the electrically inert component of ultrathin and ultra-flexible neural implants. [2] This study explores the surface functionalization of BPDA-PDA with the anti-inflammatory drug dexamethasone (DEX): the innovative approach of this study is to consider the polymer as a drug delivery platform, rather than including the drug over the electroactive surface area (as done so far). In this work, DEX is covalently bound to BPDA-PDA, enabling its sustained release over a period of at least 9 weeks, useful to mitigate the FBR onset. The synthetic pathway consists in modifications of the polyimide surface, to permit the attachment of DEX. The key step is represented by the silanization process, achieved by using the commercially available triethoxysilylpropyl succinic anhydride silane (TESPSA): the succinic anhydride acts as an anchor site for the insertion of one-to-two molecules of DEX, through the formation of an ester bond. The presence of the covalently incorporated drug was confirmed by ATR-FTIR and SEM/EDX analyses. In addition, HPLC was used to evaluate the kinetic of release (expected to occur by ester bond hydrolysis between DEX and the underlaying Si-functionalized polyimide) as well as the overall drug loading. In vitro assays demonstrate that this strategy reduced pro-inflammatory markers in macrophages. In addition, the biocompatibility of the functionalized material is ensured evaluating the viability of dorsal root ganglia (DRG) neurons. Moreover, in vivo implantation in rats of the DEX functionalized BPDA-PDA substrates shows a significant reduction in inflammatory cell infiltration and fibrotic capsule thickness formed around the devices. All these data successfully demonstrate the capability of the functionalized material to mitigate FBR, for a longer durability of the devices after implantation. Overall, the covalent approach proposed in this study represents a promising strategy to improve the challenge of long-term functionality not only of peripheral nerve interfaces, but also of neural devices in general.

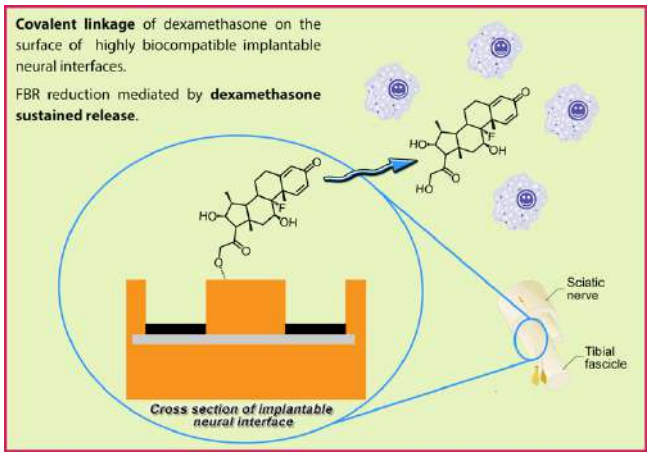

Figure 1: Polyimide BPDA-PDA surface is covalently functionalized with DEX. This neural device is implanted in the sciatic nerve of rats and the anti-inflammatory response during weeks is evaluated. This strategy demonstrated to reduce pro-inflammatory markers in macrophages and the FBR are mitigated for at least 8 weeks.

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Design of bio-responsive nanomaterials as controllable carriers and diagnostic tools

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The advancements in nanotechnology have opened new opportunities in the biomedical field, offering new strategies to achieve controlled drug delivery and to improve the diagnosis of diseases. The design of sophisticated nano-based responsive nanocarriers is driven by the need to enhance therapeutic efficacy addressing key medical challenges such as on-demand treatment and early diagnosis. My research focuses on developing nanomaterials that are responsible for targeted biomolecules or specific physiological conditions that allow achieving i) responsive drug release systems enabling the spatiotemporal controlled release of the therapeutic compound limiting side effects; ii) sensitive and selective diagnostic tools that allow the prompt intervention/prevention of the disease.
During this presentation, I will walk you through the activities related to the pH- and light-controllable drug delivery systems [1,2] that led me to the design of the recently started MSCA project, BREVERY. Within this project, my objective is to improve the brain delivery of neuroactive compounds by developing a responsive nanomaterial that overcomes the major hurdles encountered so far: i) poor brain accumulation, ii) unfavorable drug release, and iii) limited biocompatibility. To this end, I proposed the develop a novel dual-stimuli responsive, biocompatible nanomaterial enabling the spatiotemporal controlled release of drugs to the brain. Such a versatile biocompatible multi-drug nanocarrier will pave the way toward a new era for the treatment of neurodegenerative disorders (i.e. Alzheimer's and Parkinson's diseases, etc.), with a remarkable impact on society and healthcare systems worldwide.
Finally, I will present stimuli-responsive nanomaterials specifically designed to improve the diagnosis of bacterial infections, with the final aim of improving their treatment and limiting the spread of antimicrobial resistance, which is one of the most concerning health problems worldwide, causing millions of deaths and hospitalizations each year. In particular, I will focus on fluorescent-based indirect approaches to sensitively and selectively detect bacteria (i.e. *S. aureus* and *K. pneumoniae*), showing the results achieved with specifically designed nanomaterials enabling the cost-efficient indirect detection of bacteria. [3,4] Alternatively, the nanomaterials are specifically designed to enable their incorporation into biocompatible hydrogels and thus achieve wearable sensors for the diagnosis of infected wounds. [5]
These responsive nanomaterials will contribute to developing personalized medicine and point-of-care diagnostics, supporting the decentralization process of healthcare systems worldwide and tackling some of the most concerning health threats such as neurodegenerative disorders and bacterial infections.

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Bioinspired Nanocrystalline Surfaces for Bone Infection Treatment: Exploring the Osteoinductive and Optimized Properties of Antibacterial Nanostructures

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Keywords: Antibacterial nanostructured surface, antibiotic resistance, calcium phosphate, bioinspired material, calcium phosphate

Antibacterial Nanostructured surfaces (ANSs) have recently emerged as an innovative approach to treat antibiotic-resistant infections, one of the major threats to public health on a global scale. [1] Their action relies in their micro-nanotexture, which triggers a contact-killing mechanism of bacteria. [2] However, ANSs are commonly made of silicon, metal, polymer and other bioinert materials, facing lack of bioactivity as a challenge. [3] Bioactivity constitutes an essential quality for inducing tissue regeneration and it is particularly critical for bone regeneration, a calcium phosphate-constituted hard tissue. [4] Therefore, this study investigates the synthesis and properties of novel and bioactive calcium phosphate nanocrystalline ANSs, characterized by ordered arrays of nanostructures with enhanced osteogenic properties and their modulation by doping agents.

The ANSs were produced starting from an amorphous calcium phosphate (ACP) substrate via a bottom up, biomineralization-inspired crystallization. The physicochemical and structural properties of ANSs were studied by various analyses including SEM, XPS, FTIR and TEM. The antibacterial activity was tested against antibiotic-resistant strains of Gram-negative (*P. aeruginosa)* and Gram-positive (*S. aureus*) bacteria, common pathogens in chronic wound infections, such as bone infections. An additional assessment of the oxidative stress induced by ANSs was performed on bacterial and mammalian cells (renal epithelial cell line of VERO E6 monkey). Finally, the biocompatibility and osteogenic potential of the ANSs were studied on mammalian and human adipose-derived mesenchymal stem cells (Ad-MSCs).

The ANSs produced in this study exhibited important antibacterial properties, coupled with biocompatibility and bioactivity on mammalian cells as well as Ad-MSCs. Remarkably, bacteria adhering to these nanosurfaces showed a mortality rate of up to 75% after only 4 hours of contact. These bioinspired ANSs displayed a nano-topography of crystal nano-needle arrays (Figure 1), modulated through the incorporation of osteogenic ions (i.e. Zn, Cu) and molecules (i.e., citrate). Interestingly, the nano-topographies with thinner and less organised nanoneedles correlated with a higher antibacterial effect, probably related to mechanical tearing of the bacterial cell and wall membrane and induction of oxidative stress. In contrast, thicker and more organized nano-needles presented higher compatibility and osteoinduction on Ad-MSCs, which showed higher proliferation and differentiation.

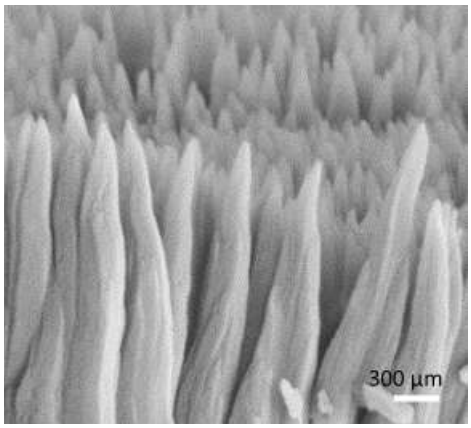

Figure 1: SEM image of nano-needle topography of bioinspired antibacterial nanostructures surfaces (ANSs).

In conclusion, this research provides a promising approach for the development of an effective treatment for antibiotic resistant infections, through advanced and bioinspired calcium phosphate ANSs. These surfaces exhibit relevant bactericidal activity against *P. aeruginosa* and *S. aureus*, while exhibiting enhanced osteogenic properties, particularly on Ad-MSCs cells. The refinement of these nanosurfaces paves the way for the development of clinically relevant materials capable of both fighting chronic infections and stimulating the regeneration of bone and other calcium phosphate tissues.

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Strategies and technological approaches to reveal proteins in traces from plasma samples

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Keywords: Proteomics, LC-MS/MS, targeted proteomics, plasma, protein fractionation

Plasma is a highly complex biological matrix, characterized by a wide dynamic range of protein concentrations. A few highly abundant proteins—such as albumin, immunoglobulins, fibrinogen, and complement proteins [1] —can mask the presence of low-abundance proteins, many of which are potential biomarkers for disease diagnosis and progression. Identifying and quantifying these proteins in trace amount remain a significant analytical challenge.
In this study, we explored and optimized various combinatorial strategies to reduce plasma complexity prior to mass spectrometry analysis. The approaches include fractional precipitation, cryoprecipitation, [2] ion-exchange fractionation, and combined proteolysis steps. [3] These workflows aim to enrich low-abundance proteins while minimizing the loss of targets of interest, a common risk when multiple purification steps are applied. Additionally, we focused on avoiding the use of commercial kits for high-abundance protein depletion, which, although effective, can be costly and introduce unintended protein loss.
Our research is currently focused on two main topics. The first is the identification and quantification of the oxidation state of von Willebrand factor (vWF), a low-abundance plasma protein (~10 µg/mL), which may serve as a risk marker for thrombotic events under conditions of high oxidative stress. [4] The second is the detection of proteins secreted by the intestinal microbiota that may translocate into the bloodstream under pathological conditions such as inflammatory bowel disease (IBD), where increased intestinal permeability has been reported. [5]
Preliminary results highlight the importance of tailoring sample preparation strategies based on the biochemical nature of the proteins of interest, as well as on the final analytical sensitivity required. This work contributes to the ongoing development of reliable protocols for proteomic profiling in clinical research, particularly in the context of biomarker discovery.

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A Statistical Approach towards Biofilms Hydrophilicity Characterization integrating ANOVA, DOE, and PCA.

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Keywords: bioplastics, starch, hydrophilicity, ANOVA, chemometrics,

Biofilm hydrophilicity is critical in the development of starch-based bioplastics, yet conventional gravimetric methods for assessing moisture content (MC), water solubility (WS), swelling degree (SD), and water vapor absorption (WVA) are plagued by high intrinsic variability and inconsistent experimental conditions. In this study, we present a robust statistical framework that integrates univariate analysis (ANOVA) with multivariate approaches, specifically Design of Experiments (DOE) and Principal Components Analysis (PCA), to accurately characterize the hydrophilic properties of starch/glycerol/carboxymethylcellulose (CMC) films. By systematically varying film composition within a defined pseudocomponent domain via a Simplex Centroid Design, seven distinct formulations were synthesized. [1] Six of the compositions were employed as a training set to develop Multiple Linear Regression (MLR) models for each hydrophilicity parameter, while the seventh composition served to validate these models.
Statistical analysis revealed that the variability introduced by film preparation and gravimetric procedures is significantly lower than the effects driven by compositional changes. Films with reduced starch content exhibited increases in both MC and WVA, whereas WS decreased with higher glycerol and CMC levels; notably, SD was largely governed by the CMC concentration, showing a pronounced increase in CMC-rich formulations. Response surfaces constructed from the MLR models, shown in Figure 1, effectively depicted these trends.

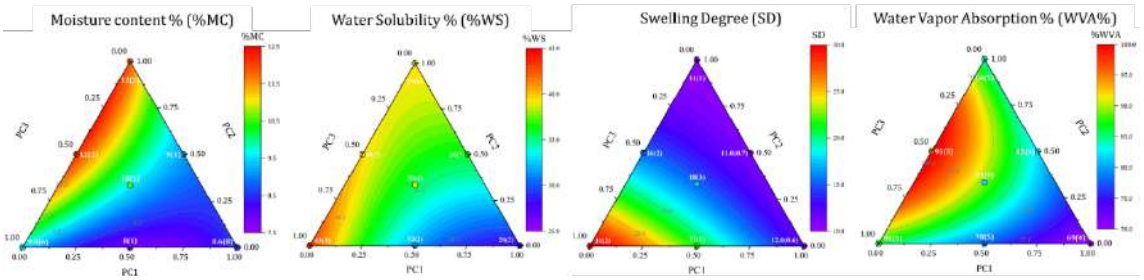

FIGURE 1: Computed response surfaces for Moisture Content (%MC), Water Solubility (%WS), Swelling Degree (SD) and Water Vapor Adsorption (%WVA) within the pseudocomponents domain and comparison with the experimental values

Lastly, PCA was run as multicriteria decision making tool to further condensed the responses under investigation. The PCA loading plots indicated strong positive correlations between WS and WVA and a moderate correlation with MC, while SD behaved independently. This approach not only improves reproducibility in hydrophilicity assessments but also offers a rational basis for optimizing bioplastic formulations.
The methodology builds on and extends previous studies [1,2] addressing notable shortcomings of traditional techniques and establishing a systematic route for material characterization. By rigorously quantifying the impact of formulation variables, the present work sets a benchmark for future research in biofilm analysis. The integration of ANOVA, DOE, and PCA ensures that both intrinsic variability and composition-dependent effects are properly accounted for, thereby facilitating the prediction of hydrophilic behavior across a wide formulation range. Ultimately, this statistically grounded approach advances the development of environmentally friendly bioplastic technologies with enhanced performance for applications in food packaging, medical devices, and beyond.

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An ABMD-based workflow for predicting relative residence times of ligands against systems of pharmacological interest.

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Keywords: molecular dynamics, unbinding kinetics, ABMD,

In silico methods for investigating ligand/target dissociation rates provide an efficient tool in drug discovery campaigns. Unbinding kinetics strongly correlate with the efficacy and safety profiles of drugs and impact their therapeutic effect. The use of molecular dynamics-based methods, enhanced sampling techniques in particular, has been widely explored to devise useful protocols and sample rare events using limited computational resources. Nonetheless, the application of such protocols to large datasets remains challenging. [1] Herein, we present an Adiabatic Bias Molecular Dynamics (ABMD)-based [2,3] workflow for predicting relative unbinding times of congeneric compounds against systems of pharmacological interest. ABMD exploits a harmonic biasing potential to reach the transition of free-energy barriers along a specific reaction coordinate (CV) during MD simulations. Once an endpoint state has been chosen, a harmonic biasing potential is applied, but only when the system attempts to recede along the coordinate. Thus, exploration is driven toward the predefined endpoint with minimal perturbation and according to thermal fluctuations of the system.
Starting from protein-ligand complexes, we investigated the application of ABMD on systems of pharmacological interest using the distance between the centers of mass of the ligand and of the binding site as the CV. We devised a semi-automatic workflow to sequentially perform the steps of preparation, equilibration, MD, and ABMD simulations, and finally the analysis of the generated trajectories. For each ligand-protein system, after equilibration, ABMD simulations in multiple separate replicas were performed. Each ABMD simulation lasted until the unbinding event was observed or until a certain ceiling simulation time was reached. The workflow was developed and tested using both the AMBER and GROMACS molecular dynamics engines.
The calculated mean times of unbinding of the ligands against their experimental kinetic rates displayed promising correlations.
The same workflow was attempted on a dataset of mutants of the muscarinic M3 receptor using tiotropium as the only ligand. In that case, the use of the more comprehensive Path Collective Variables (PCVs) [4] was necessary to obtain favorable results. Simulations on other systems of pharmaceutical interest are also currently on-going.

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New Eu(III)-based luminescent probes for time-gating fluorescence microscopy

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Keywords: Lanthanide emission, fluorescent probes, immunostaining.

The emission of trivalent lanthanide ions (Ln^{3+}) is characterized by a long lifetime (up to milliseconds), which is advantageous in the field of tissue and cell fluorescence microscopy. By using time-gated techniques—i.e., acquiring the image after the background fluorescence has faded out—it is possible to record images with enhanced quality, maximizing the signal-to-background ratio. [1]
Generally, the Ln^{3+} emission is achieved through an indirect excitation, where the metal center is coordinated with a chromophoric ligand (antenna) that absorbs light and transfers energy to the lanthanide ion (antenna effect). As a result, Ln^{3+} complexes exhibit negligible self-absorption and a significant difference between absorption and emission wavelengths. With a careful selection of ligands and Ln^{3+} ions, emission can be observed in both the visible and near-IR regions. [2]
We prepared two Eu^{3+}-based red-emissive probes that can be used to stain either antibodies for immunofluorescence or drug-delivery cyclodextrin-based (nano) materials. To achieve this, we designed two ligands that are able to coordinate a lanthanide ion, sensitize its emission, and interact with the desired target.
In one case, we modified a pyridine dicarboxylic acid core by extending its conjugation and decorating its backbone with a bioconjugable unit that reacts with the nucleophilic sites (i.e., lysine) of antibodies and proteins. In the other case, we introduced an adamantyl unit onto a β-diketonate ligand to facilitate its inclusion within the cavities of cyclodextrin-based materials. In the presence of a Eu^{3+}-containing buffer (pH 7.4), the ligands and the stained systems exhibit a 615 nm centered emission, associated with a lifetime of *ca.* 300 μs.

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Next-Generation FDDNP Analogues for Amyloid β Visualization

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Keywords: Alzheimer's disease, amyloid β detection, fluorescent probes

Fluorescent probes play a critical role in biomedical imaging, particularly in the study of neurodegenerative diseases such as Alzheimer's disease (AD). Characterized by the accumulation of amyloid β (Aβ) aggregates, AD demands highly sensitive and selective diagnostic tools for early detection. [1] In recent research, Prof. Dr. Košmrlj and colleagues synthesized and characterized 21 novel fluorescent probes designed for Aβ aggregate targeting. Among these, probe 1Ae (Figure 1) exhibited large Stokes shifts, high quantum yields, and low micromolar affinity for Aβ fibrils. Upon binding, the probe demonstrated a significant fluorescence enhancement, facilitating clear visualization of Aβ plaques in both cellular models and *postmortem* AD brain tissue. Computational studies further elucidated the probe's photophysical properties and binding interactions. These findings point to the potential of advanced fluorescent probes for improving AD diagnostics, paving the way for more effective early-stage detection and disease monitoring. [2]

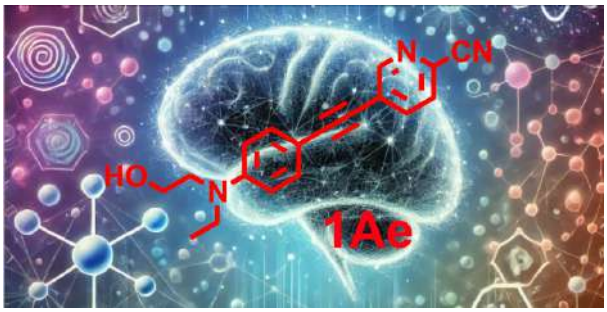

Figure 1: Structure of fluorescent probe **1Ae** for amyloid β detection.

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Figure 1 was produced by OpenAI. (2025). Image generated by DALL·E.

The role of NMR Spectroscopy for the investigation of nanocarriers in drug delivery applications

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Keywords: Drug Delivery Systems; NMR spectroscopy; thermoresponsivity; anticancer active ingredients

Clinical effectiveness of potent anticancer active ingredients is frequently hampered by challenges, such as low water solubility, susceptibility to degradation, and poor target selectivity. To overcome these limitations, Drug Delivery Systems (DDS) have emerged as a promising strategy, offering protective encapsulation, controlled release, and improved bioavailability of anticancer agents. Within this research context, Nuclear Magnetic Resonance (NMR) spectroscopy stands out as a powerful and versatile technique for the physico-chemical characterization of advanced nanosystems. In particular, NMR is suitable for the analysis and optimization of DDS, overcoming known limitations of traditional techniques. This contribution focuses on a thermoresponsive, PEGylated-fluorinated block copolymer (PEGMA-co-FA) used as a nanocarrier for Combretastatin A-4 (C-4), a potent tubulin polymerization inhibitor with anticancer activity. Detailed conformational and temperature-dependent behaviors were examined using multidimensional NMR techniques. Additionally, DOSY experiments were employed to estimate the hydrodynamic size of the nanocarriers, and the enhanced solubility of C-4 loaded in the DDS was assessed [1]. A similar approach will be extended to nanomicelles constituted by Vitamin E-TPGS and Kolliphor ELP for curcumin encapsulation, combined with a thermosensitive hydrogel for potential melanoma therapy [2,3].

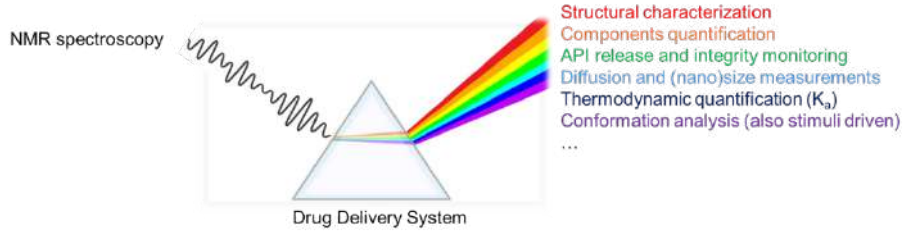

Figure 1: Example of NMR multi-analysis on drug delivery system.

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Revealing the intricate regulatory mechanisms of small Rho-GTPases

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Keywords: Molecular dynamics, QMMM, catalytic reactions, drug discovery, cancer inhibitors.

Small Rho GTPases are essential regulatory proteins in eukaryotic cells, functioning as molecular switches that cycle between an active GTP-bound state and an inactive GDP-bound state. Through GTP hydrolysis and GDP/GTP exchange, Rho GTPases orchestrate key cellular processes, including cytoskeleton remodelling, cell polarity, cell cycle regulation, and gene expression signalling pathways. This dynamic cycle is precisely regulated by two classes of proteins: GTPase-activating proteins (GAPs), which accelerate GTP hydrolysis, and guanine nucleotide exchange factors (GEFs), which facilitate GDP/GTP exchange. [1] Dysregulation of Rho GTPases has been implicated in the onset and progression of various diseases, including cancer and pathogen infections. Despite extensive biochemical and computational studies, many aspects of the activation (hydrolysis) and deactivation (GDP/GTP exchange) mechanisms of small GTPases remain poorly understood.

In this contribution, the regulatory mechanisms of Rho GTPases will be explored through atomic simulations. Specifically, we employ classical molecular mechanics and molecular dynamics (MM and MD), quantum mechanical (QM) methods, and hybrid QM/MM MD approaches, combined with advanced sampling techniques, to investigate: (i) the GTP hydrolysis mechanism of RhoA, promoted by GTPase-activating proteins (Figure1); (ii) GDP/GTP exchange mechanisms of Rho GTPases, in both wild-type and cancer-associated isoforms; [2,3] and (iii) the development of small-molecule inhibitors targeting Rho GTPases in cancer.

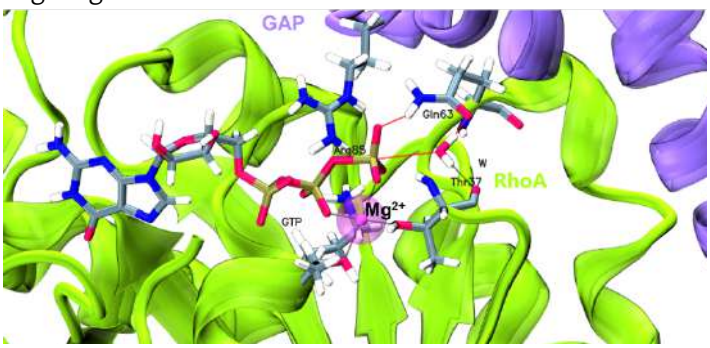

Figure 1: Zoom on the RhoA-RhoGAP complex interface, highlighting the interaction between key residues, GTP, and the nucleophilic water molecule.

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Effects of N-acylethanolamines on metabolic damage and cellular stress mechanisms driven by obesity

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Keywords: Liver, adipose tissue, metabolic syndrome, senescence, autophagy

N-acylethanolamines (NAEs) are endogenous lipids that belong to the class of non-canonical endocannabinoids and play important roles in regulating inflammation and energy metabolism. [1] It is well known that their signaling is disrupted during obesity, [2] but the pharmacological effects of NAE-based formulations in metabolic disorders remain poorly understood. At cellular level, the excessive caloric intake triggers stress-related mechanisms such as autophagy dysregulation, mitochondrial dysfunction, oxidative stress, and cellular senescence. [3] Targeting these cellular responses may offer benefits for managing obesity. We first investigated the beneficial effects of an NAE-based formulation which has a higher content of oleoylethanolamide (OEA) and lower amounts of linoleoylethanolamide (LEA), palmitoylethanolamide (PEA), and stearoylethanolamide (SEA), in improving metabolic damage in high-fat diet-fed obese mice. Our results showed that OLA treatment reduced body weight, fat mass, and insulin resistance in obese mice, while improving liver and lipid profiles. It also decreased fatty acid accumulation and restored insulin signaling in the liver. In adipose tissue, OLA promoted thermogenesis in brown adipose tissue and the beigeing of white adipose tissue.
Next, we used in vitro models to further assess the effects of individual NAEs on metabolic stress-related responses, such as autophagy dysregulation, cellular senescence induction, and associated mitochondrial oxidative damage, in different cell types involved in tissue metabolism (hepatocytes, adipocytes, and primary murine fibroblasts).
In our in vitro studies on HepG2 cells, we found that each NAE induced protective autophagy and mitophagy mechanisms, as evidenced by the modulation of the LC3-II/p62 pathway, upregulation of ATG3 and ATG7 transcription, and increased mKeima protein expression (evaluated by flow cytometry). Furthermore, NAE treatment enhanced the autophagic pathway in the presence of palmitic acid (PA), as shown by the phosphorylation of AMPK, and reduced cytotoxicity. To mimic obesity-associated cellular stress, we exposed mouse ear fibroblasts to PA, a major component of fat-enriched diets, for 7 days. Notably, PA increased senescence markers, including SA-β-galactosidase-positive cells and elevated p21/p16 expression. In contrast, NAE treatment enhanced mitochondrial respiration and downregulated p21 and IL-6 mRNA expression—two tumor suppressors associated with the senescence-associated secretory phenotype (SASP). Additionally, flow cytometry analysis demonstrated the senomorphic activity of NAEs in reducing oxidative stress linked to doxorubicin-induced DNA damage.
Taken together, NAEs and their combinations may offer a strategy to counteract metabolic alterations linked to obesity and dysmetabolism by activating protective mechanisms and reducing the senescent phenotype and associated oxidative stress.

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Exploring Cellulose Nanocrystals as Potential Nanocarriers for Copper Ion Delivery

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Keywords: Cellulose nanocrystals, Copper, chelation, DLS, zeta-potential.

Cellulose nanocrystals (CNCs) are gaining attention as sustainable and versatile nanomaterials for biomedical applications due to their high surface area, biocompatibility, and tunable surface chemistry. Extracted from natural cellulose sources, CNCs provide a biodegradable and non-toxic platform, easily functionalized through surface modifications. In particular, TEMPO-mediated oxidation introduces carboxyl groups, enhancing colloidal stability and enabling covalent attachment of biologically active ligands.[1] These features make CNCs promising candidates for advanced drug delivery and biomaterial engineering. In this context, our study explores a novel application: investigating whether CNCs can interact with and potentially serve as nanocarriers for copper ions. To address this, TEMPO-carboxylated CNCs were examined for their interactions with copper under varying concentrations and conditions using multiple analytical techniques. Furthermore, these CNCs were functionalized *via* DMTMM chemistry in water with two copper-binding agents—the tripeptide GHK and 5-aminomethyl-8-hydroxyquinoline (AMHQ) [2]. Both pristine and functionalized CNCs were characterized by dynamic light scattering (DLS) and zeta potential analysis to assess size distribution and surface charge, as well as by FT-IR spectrophotometry and atomic force microscopy (AFM) to evaluate chemical modifications and morphological features. The interaction between copper ions and CNCs was further investigated using UV-Vis titrations at pH 7.4 and radiolabeling with Copper-61 so to better assess the potential of CNCs as nanocarriers for metal ion delivery aimed at inducing cuproptosis [3].

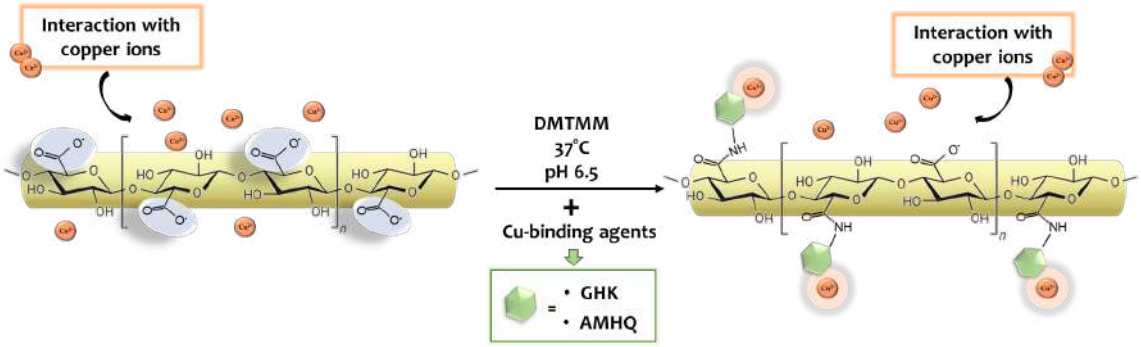

Figure 1: Schematic of Cu^{2+} interaction with: *i)* pristine CNCs (left), and *ii)* GHK/AMHQ-functionalized CNCs (right).

Acknowledgements

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Design and synthesis of linear polyamines for the development of highly performing ferritin-conjugates

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Keywords: RNA interference, gene therapy, polyamines, bioconjugation

Cancer is one of the most health and socioeconomic disease worldwide. Despite significant progresses have been registered, and several drugs are available to treat cancer, the onset of resistance is a major drawback that often causes the failure of the treatment. [1] Recently, new therapies based on small interfering RNA (siRNA), micro-RNA mimics (miR mimics), and antisense micro-RNA (antagomiR) have been largely exploited to cure cancer, neurodegenerative diseases, and some rare diseases. [2] In the last decade, to circumvent the obstacles imposed by delivery vehicles such as nanoparticles which are composed of potentially toxic exogenous materials, the development of protein-based systems (HumAfFt) as well as chemical modifications of canonical oligoribonucleotides have been considered as promising strategies to improve RNA delivery, stability and efficacy. In a previous work, RNA encapsulation in HumAtFt was achieved by means of poly-cationic molecules, namely polyamines (PAs), which can both specifically bind to the protein and stabilize the oligonucleotide through electrostatic interactions. Results obtained with cyclic PAs highlighted an efficient siRNA delivery and a good silencing action on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression in three cancer cell lines. [3] Recently HumAfFt-PAs system has been also employed for the successful delivery of miRNA [4] and for the encapsulation of PNAs. In order to evaluate a structure-encapsulation relationship between PAs and oligonucleotide, a library of linear polyamine molecules (**R-PA-2.2**) (Figure 1) was rationally designed and synthesized, featuring a pentafluorobenzene sulfonamine moiety to ensure the anchorage of the polyamines to the cysteine residues of HumAfFt. Unlike cyclic amines whose synthesis relied on subsequent reductive amidation reactions starting from *N*-methyl-4-piperidone and final introduction of the pentafluorobenzene moiety, linear PAs are synthesized by performing subsequent Steglich couplings to join glycine, β-alanine, and methylamine as major building blocks of this series with further introduction of the selective moiety for thiols.

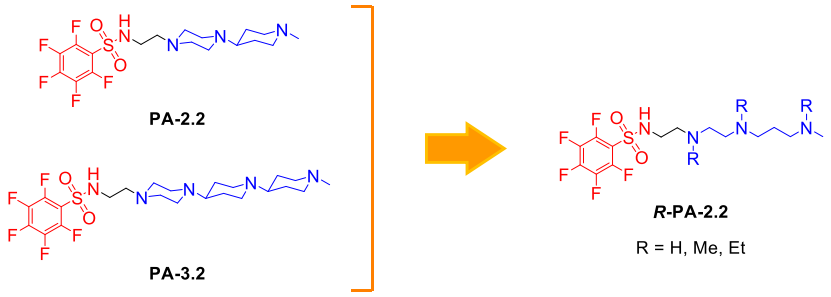

Figure 1: Design and chemical structure of linear polyamines ***R*-PA-2.2**.

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Fluorinated oxadiazoles as potential scaffolds for biomaterials

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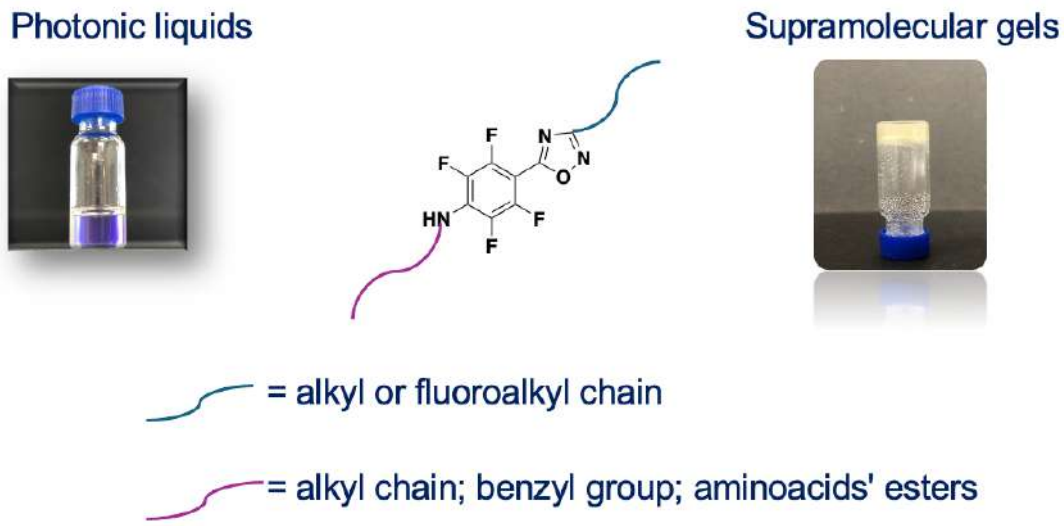
Keywords: fluorinated molecules, oxadiazole, supramolecular gel, photonic liquids.

Photonic liquids and gels have been widely used in several application fields among which biomedical applications, thanks to their intermediate behaviour between solid and liquid. [1,2]
The research of new chemical scaffolds able to self-assemble, hence giving rise to smart materials is still challenging. For example, a class of fluorophenyl-oxadiazoles has been proved to behave as organogelator while several fluorinated triazole salts showed ionic liquid crystals properties. [3,4] in this framework, 1,2,4-oxadiazole bearing fluorinated moiety was used as core for the synthesis of new class of molecules taking advantage from its great tunability.



Scheme 1: Fluorophenyl-1,2,4-oxadiazole based molecules behaving as fluorescent liquid crystals or supramolecular gels in dependence of the substituents.

Fluorophenyl-1,2,4-oxadiazoles based molecules differing for substituents on phenyl ring (aliphatic, aromatic or aminoacid derivatives) and on heterocycle (fluorinated or alkyl chain) were synthesized and studied as potential molecules for self-assembly. Chemical-physical properties of the compounds were drastically influenced by structural modification on the core, behaving as photonic liquids or as organogelators. Both type of materials can be of great interest for biomedical applications.

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Biodegradable Organic Smart Nanoparticles for Biomedical Applications on Earth and Beyond

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Keywords: Stimuli-responsive nanomaterials; Piezoelectric nanoparticles; Photothermal stimulation; Magnetic nanoparticles; Biodegradability

Our recent work in nanomedicine field has focused on shifting from inorganic, stimuli-responsive nanomaterials to biodegradable and highly biocompatible alternatives, aiming to enhance clinical translatability. This transition is particularly relevant in the development of piezoelectric, photothermal, and magnetothermal nanoplatforms, which show great potential for biomedical applications.

In this framework, we have extensively explored piezoelectric nanoparticles due to their ability to generate localized electrical stimuli under mechanical stress, opening new perspectives for remotely controlled therapeutic strategies. Our first demonstration of piezoelectric activation using tetragonal barium titanate nanoparticles revealed their potential for wireless neuronal stimulation. [1] To improve biocompatibility and degradability, we have recently moved towards polymeric alternatives, such as PVDF-TrFE nanoparticles. [2] We successfully used these ultrasound-responsive, drug-loaded piezoelectric nanoparticles for glioblastoma treatment, achieving a combined anticancer effect of chemotherapy and localized electrical stimulation. Further advancements in our work include the development of chitosan-based biodegradable nanoparticles exhibiting significant piezoelectric properties. [3] We have now successfully demonstrated their ability to respond to ultrasound and induce antiproliferative and pro-apoptotic cues in patient-derived glioblastoma spheroids using an innovative *in ovo* model.

Another major focus of our research is on polydopamine nanoparticles (PDA NPs), which possess intrinsic antioxidant activity and near-infrared (NIR) photothermal responsiveness. Our studies have demonstrated that PDA NPs are highly effective as neuroprotective agents, mitigating oxidative stress-related neuronal damage. Additionally, we have exploited PDA NPs as photothermal agents capable of modulating neuronal activity via NIR light-induced intracellular heating. [4] These multifunctional properties make PDA NPs promising candidates for neurodegenerative disease treatment. As part of the *Prometeo* project on the International Space Station, we are currently investigating their potential to counteract oxidative stress-induced neurodegeneration, a condition relevant both in terrestrial pathologies such as Parkinson's disease and in space environments, where microgravity and ionizing radiation contribute to oxidative damage.

Finally, the development of organic magnetothermal nanoparticles remains an open challenge. While iron oxide nanoparticles have demonstrated efficacy in hyperthermia treatments, [5] transitioning to organic alternatives (*e.g.*, ferritin nanoparticles) is hindered by their low magnetic moment.

In conclusion, the evolution of nanomedicine towards biodegradable, responsive, and biocompatible nanoplatforms marks a significant step forward in the translation of these technologies into clinical practice, with potential implications extending beyond Earth's boundaries.

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Synthetic DNA Transducers: Enhancing CRISPR-Cas12a Applications Beyond Nucleic Acids

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Keywords: CRISPR, Cas12a, PAM, DNA Transducer, DNA repair enzyme.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has revolutionized molecular diagnostics, renowned for its precision, efficiency, and adaptability. While traditionally employed for nucleic acid detection, recent advancements have expanded CRISPR utility to encompass non-nucleic acid targets, addressing previous limitations in detecting diverse biomolecules and offering new avenues for diagnostics and therapeutic monitoring. [1] Here, we present strategies that harness CRISPR programmability alongside DNA nanotechnology to establish artificial communication between CRISPR-Cas proteins and non-nucleic acid targets, facilitating probing, processing, and conversion of target recognition into an amplified CRISPR-based signal.

Specifically, we introduce a novel class of Cas12a regulators termed PAM-engineered Toehold Switch DNA, featuring a re-engineered locked protospacer adjacent motif (PAM) within the loop (Figure 1A). This design enables precise control of Cas12a activity in response to specific molecular targets via structure switching and PAM complementation. The reconfiguration of PAM-engineered Toehold Switch DNA is governed by a proximity-based reaction network, facilitating single-step detection of diverse targets, including IgG antibodies, small molecules, and microRNAs, with high sensitivity and specificity, even within complex matrices. [2] Additionally, we developed a strategy for monitoring DNA repair enzyme activity, exemplified by mono- and bi-functional DNA glycosylases (e.g., UDG, hOGG1). This method integrates an activity-based approach with Cas12a-assisted signal amplification. It utilizes a lesion-containing DNA transducer that undergoes structural reconfiguration upon lesion-excision-driven DNA repair activity, triggering Cas12a activity and fluorescence signal transduction (Figure 1B). The resulting CRISPR-based cascade reaction enables highly sensitive, single-step monitoring of various DNA repair activities in cell lysates and facilitates high-throughput screening of repair enzyme inhibitors, underscoring its potential as a versatile tool for clinical diagnosis and drug discovery.

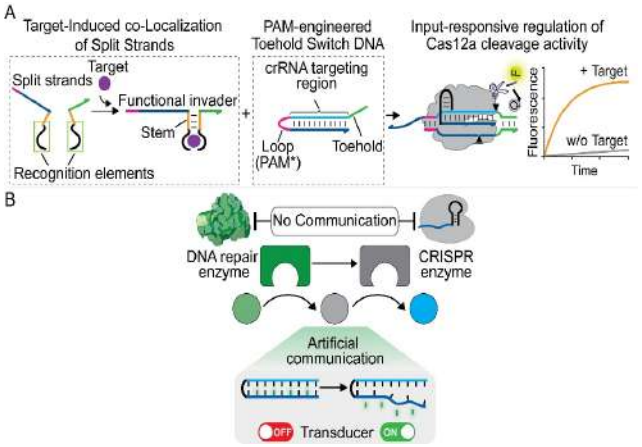

Figure 1. (A) PAM-engineered Toehold Switch DNA reconfiguration enables precise control of Cas12a activities in response to specific molecular targets via structure switching and PAM complementation within a proximity-based reaction network. (B) Schematic of the artificial communication pathway between DNA repair and CRISPR-Cas12a enzymes controlled by a DNA-based molecular transducer.

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Novel Dual-Targeting Carbonic Anhydrase Inhibitors: A Promising Strategy for Cancer Therapy

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Keywords: Cancer, Dual-targeting carbonic anhydrase inhibitors, Wnt/β-catenin signalling, Heterocyclic compounds, Anticancer therapy.

Carbonic anhydrases (CAs) are ubiquitous metalloenzymes that catalyse the reversible conversion of carbon dioxide and water to bicarbonate and a proton, *via* a coordinated metal ion. CAs activity is pivotal to metabolism regulation under both physiological and pathological conditions, [1] since these enzymes are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate, CO_2 homeostasis, intra and extracellular pH balance and tumorigenesis. Given their involvement in several diseases, including cancer, human CA (hCA) isoforms have gained significant attention as potential therapeutic targets for the development of novel anticancer agents. [2]

Herein, in an effort to identify novel carbonic anhydrase inhibitors as anticancer agents, we designed and synthesised two series of compounds by strategically modifying the scaffold of our previously reported tubulin assembly inhibitors. [3] Therefore, in both series, a sulfonamide moiety has been introduced at different positions on the *N*1-phenyl ring. Additionally, the pyrrole-based derivatives were substituted at C3 and C4 positions, whereas the indole-based analogues were decorated at C3 position. Among the tested derivatives, two lead candidates, **1** and **2**, emerged as particularly promising due to their potent inhibition of hCA IX and XII isoforms, which are involved in cancer (Figure 1). [4] Notably, both compounds exhibited cytotoxic activity comparable to 5-FU against HCT116 cells and demonstrated superior potency against SW480 and SW620 colorectal cancer cell lines. In addition, compound **1** strongly inhibited the MDA-MB 231 and BT-549 triple-negative breast cancer cell lines and was superior to the reference compound SLC-0111. Molecular modelling studies further supported these findings, revealing docking poses within the binding sites of four hCA isoforms that were consistent with the observed K_i values.

Noteworthy, compound **1** proved to be a novel dual-targeting agent, effectively inhibiting both hCA and the Wnt/β-catenin signalling pathway. Moreover, it showed potential in overcoming drug resistance by restoring sensitivity in P-gp expressing cell lines. These results highlight **1** as a lead compound of a novel class of dual-targeting carbonic anhydrase inhibitors with broad-spectrum anticancer potential, paving the way for further studies to explore its therapeutic applications in cancer treatment.

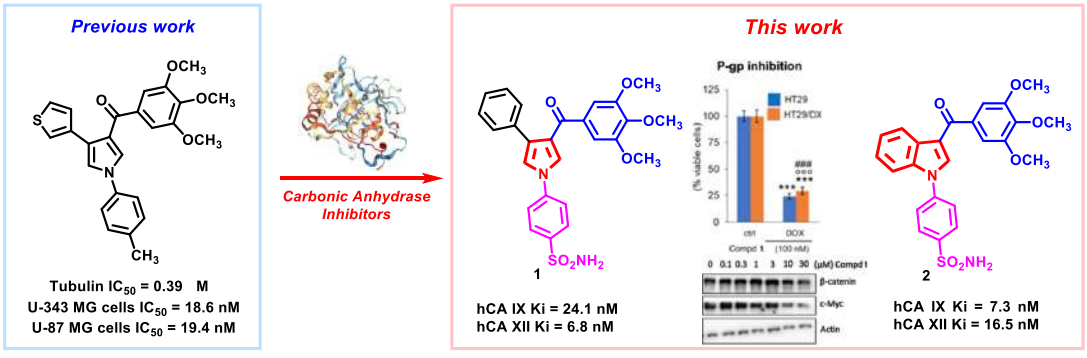

Figure 1: Rational behind this work. Structures of compounds **1** and **2**. Inhibition of both P-gp and Wnt/β-Catenin signalling pathway by compound **1**.

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Evaluation of rationally designed Topoisomerase inhibitors and FLASH radiotherapy for the treatment of head and neck carcinoma

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Introduction. Locally advanced head and neck carcinoma (HNSCC) continues to have severe morbidity with less than 50% long-term survival despite the advancement in the available therapies. Depending on the stage of the disease, the treatment approach can involve concurrent chemoradiotherapy (CRT) but both the drug of choice (cisplatin) and radiotherapy (RT) are associated with systemic toxicities. This highlights the need of new strategies to improve the patient outcomes. [1] On this regard: i) topoisomerase are an increasingly interesting targets in cancer therapy as the effect of their inhibition has been already demonstrated for the treatment of several neoplasms, [2] while ii) FLASH-RT is emerging as a replacing strategy to conventional RT due to the sparing effect on healthy tissues. [3] Thus, the exploration of new classes of topoisomeserase inhibitors, and their potential association with FLASH-RT may pave the way for the development of new therapeutic strategies to overcome the clinical limitations of conventional CRT in the management of HNSCC. Results. Our findings demonstrated a significant cytotoxic effect of a novel topoisomerase inhibitor (ARN24139), with an IC^{50} value of ~7.29 µM within 48 hours on SCC-25 2D cells. The antitumor activity of ARN24139 has been confirmed by clonogenic assay, wound-healing assay, and detection of DNA damage. The progressive decrease in colony formation at increased concentration of the compound demonstrated the efficacy of ARN24139 in reducing cancer cell division. A significant increase in double strand breaks was observed with a concentration below the IC^{50} and immediately 24h post-treatment with a gradual decrease within 72 hours, probably due to the activation of DNA damage repair pathways. Viability assays performed in three-dimensional tumor spheroids (3D) showed a significant decrease in cell survival by reaching an IC^{50}_{48h}: ~12.64 and a severe loss of spheroid integrity. The synergism between ARN24139 and FLASH-RT has also been investigated with different orders of administration and duration of treatment. A constant concentration of 5 µM-ARN24139 has been tested with increased radiation doses (0, 4, 8, 12, 16 Gy) and viability monitored for up to 8 days. The best synergistic effect was observed when the compound was associated with low doses of RT, compared to the respective monomodal treatments. Finally, alternative in vivo models were used to further corroborate the in vitro findings. Conclusion. These results demonstrate: i) the encouraging efficacy of ARN24139 over the gold standard, and ii) its synergistic effect in association with FLASH-RT at low irradiation doses. Therefore, the combination of topoisomerase inhibitor with FLASH-RT may result in a promising approach showing an enhanced therapeutic *vs.* side effects equilibrium for the management of head and neck squamous cell carcinomas.

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A High-Sensitivity approach for detection of breast cancer-derived extracellular vesicles using single molecule array technology

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Keywords: Breast cancer, extracellular vesicles, Single Molecule Array

Introduction: Extracellular vesicles (EVs) are small membrane-bound particles released by cells that carry a variety of molecular contents such as proteins, lipids, and nucleic acids. These vesicles play crucial roles in cell-to-cell communication and can reflect the physiological and pathological state of their cells of origin. Furthermore, EVs are considered promising in cancer research due to their carriage of tumour-related molecules. [1,2] However, current methods for quantifying EVs often lack the sensitivity and specificity to distinguish them from those derived from normal cellular processes. [3] Additionally, markers from cancer cells are present at significantly lower concentrations compared to those found on general EVs and a sensitive and easy-to-use method is needed to prove their real clinical potential. [2]

Methods: Single Molecule Array (SiMoA) technology, a new ultrasensitive digital ELISA immunoassay, emerged as a powerful tool for the sensitive detection of biomarkers at single-molecule level directly in unprocessed plasma [4]. SiMoA immunoassays were established based on the use of magnetic beads conjugated with an anti-CD63 capture antibody to specifically isolated EVs, followed by the use of biotinylated anti-CD9 or anti-HER2 detector antibodies to identify general and tumor-specific EVs populations. [4,5] Additional SiMoA assays were developed and tested based on the use of magnetic beads conjugated with a membrane sensing peptide (MSP) able to bind EVs for their curvature. [6,7] The results obtained with the new MSP-beads coupled with the two detector antibodies previously described were thus compared with the one obtained using traditional antibody based beads.

Results: Two SiMoA assay platforms (anti-CD63 and MSP-conjugated beads) were comparatively evaluated for their sensitivity in detecting EVs isolated from breast cancer patient-derived organoids (PDOs). Both assays demonstrated clear dose-response relationships to the increasing amounts of EVs. Furthermore, the MSP-based assay also demonstrated significantly enhanced sensitivity and superior overall analytical performance compared to the CD63 assay in distinguishing tumor-related EVs from cancerous PDOs.

The two detection strategies were also tested directly on human plasma samples collected from metastatic breast cancer patients (MBC, n=49), including 17 HER2+ and 32 HER2- metastatic patients, and healthy controls (HC, n=30). Results obtained from both assays showed that HER2-expressing EVs can be detected by SiMoA at very low concentrations in patients' plasma and that HER2+ EVs are enriched in HER2+ MBC patients compared to HER2- patients but do not differ from levels detected in HC.

Conclusion: This study demonstrates the feasibility of SiMoA technology for detecting low-abundance cancer-released EVs biomarkers, with its high sensitivity and highlight how the use of magnetic beads conjugated with a MSP can further improve the specificity of the assays.

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Fully 3D-printed electrochemical sensor for miRNA detection in liquid biopsy applications

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Keywords: 3D printing, electroanalysis, conductive PLA, miRNA, diagnostics

The demand for robust and precise point-of-care devices is particularly high in personalized medicine, where rapid and accurate detection of biomarkers is crucial for tailored healthcare and well-being. [1] In this context, electrochemical sensors offer significant advantages due to their sensitivity, specificity, and adaptability. [2] The emergence of 3D printing technology has further expanded their potential by enabling the creation of innovative electrochemical well designs, making them suitable for a wide range of applications in electrochemical sensing. [3] This study aimed to develop a fully 3D printed electrochemical sensing device featuring a three-electrode system fabricated from conductive printing materials and incorporating a microwell as the sensing platform. The assay principle of a robust electrochemical screen-printed sensor [4] was adapted to this platform, integrating a well structure to enhance fluid control and enable the detection of a microRNA biomarker associated with lung cancer. The detection mechanism was based on the hybridization of the target miRNA with an immobilized DNA probe labeled with methylene blue as a redox mediator. The sensor was thoroughly characterized and optimized, achieving a dynamic detection range of 0.001 to 400 nM and a lower limit of detection in the picomolar range, surpassing conventional screen-printed sensors. Furthermore, the sensor demonstrated high selectivity for the target miRNA over other sequences, confirming its specificity. These findings highlight the potential of 3D printing technology in advancing personalized diagnostics and prognostic healthcare solutions, ultimately contributing to improved patient outcomes and well-being. The successful integration of this sensor into clinical settings could significantly enhance diagnostic capabilities and medical decision-making by providing a reliable, cost-effective, and customizable sensing platform for detecting critical biomolecules such as miRNAs.

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pH-responsive metal-coordinated assemblies for methotrexate delivery in cancer therapy

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Keywords: metal-complexes, solution thermodynamics, drug delivery, pH-controlled release, anticancer activity

Methotrexate (MTX), a methylated folic acid derivative, is widely used to treat various malignancies. [1] However, its ability to inhibit key cellular functions leads to a lack of selectivity for neoplastic cells, causing severe side effects. Poor solubility, short half-life, and drug resistance further limit its efficacy. To address these challenges, research has focused on drug delivery systems that enhance bioavailability and targeted delivery. [2] Polymer-based systems have received particular attention due to their peculiar properties and relatively easy functionalization to respond to specific stimuli. [3]

Given the pH difference between healthy and cancerous cells, we propose the development of pH-responsive metal-coordinated assemblies containing MTX, biologically relevant metal ions (Cu^{2+} or Zn^{2+}) and polyacrylic acid (PAA), a polymer responsive to pH changes. These assemblies aim to improve MTX bioavailability, targeted delivery, and controlled release.

The assemblies were designed by leveraging the binding properties and the energetics of interactions among the system components. MTX and PAA, both negatively charged at physiological pH, interact efficiently only via metal ion coordination. The quantitative analysis of species, binding affinity, and solution thermodynamics was conducted at 25 °C and physiological pH using isothermal titration calorimetry. The binding of MTX to polymer-metal complexes at the solid-liquid interface was examined via quartz-crystal microbalance with dissipation monitoring. Results confirmed MTX adsorption onto the polymer-metal layer and its pH-dependent release. [4]

Further characterization involved dynamic light scattering, zeta-potential, and scanning electron microscopy.

In vitro cytotoxicity was assessed in U87 glioblastoma cells line, revealing enhanced anticancer activity of the metal-coordinated assemblies over free MTX. In view of a potential biological application, a preliminary investigation of the ability of the MTX assemblies to interact with human serum albumin in solution was also carried out.

Overall, the study of multiple equilibria enabled the rational design of MTX-based metal complexes that improve drug loading and controlled release. Biological assays recommend these assemblies as promising candidates for targeted MTX delivery in cancer therapy.

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Nutriosomes as Antioxidant Carriers: A Comparative Study of Cannonau Grape Pomace Extract in a Parkinson's Disease Mouse Model

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Keywords: nutriosomes; grape pomace; controlled release; Parkinson's disease; antioxidant

Grape pomace, the major side-stream of winemaking, is a rich and sustainable source of bioactive phytochemicals, particularly polyphenols, which have demonstrated potent antioxidant and neuroprotective properties. However, the clinical application of such compounds is limited by their poor aqueous solubility, instability in gastrointestinal environments, and low permeability across biological membranes. [1] Nanotechnological strategies such as vesicular carriers have been proposed to overcome these challenges. Among these, nutriosomes, phospholipid vesicles enriched with Nutriose® FM06, have shown promise as oral delivery systems, ensuring improved stability, controlled release, and enhanced bioavailability of plant extracts. [2]

This study investigated the efficacy of nutriosomes loaded with Cannonau grape pomace extract in counteracting oxidative stress and neurodegeneration in a Parkinson's disease mouse model induced by subacute administration of MPTP. The study builds upon previous evidence demonstrating neuroprotective effects of nutriosomes incorporating Nasco grape pomace extract. Notably, Cannonau extract lacks procyanidin B2, a compound present in Nasco and associated with neuroprotective functions.

Nutriosomes were formulated via sonication by hydrating phospholipid (S75), Nutriose® and Cannonau extract. They were characterized by dynamic light scattering and electrophoretic mobility. Results revealed mean diameters of ~157 nm and ~198 nm for 5- and 10-nutriosomes, respectively, with narrow size distributions and highly negative zeta potentials (–51 to –41 mV), indicating colloidal stability. Entrapment efficiency ranged from 77% to 85%. Release studies confirmed a sustained release profile over 72 hours, contrasting with the rapid dissolution of extract in free suspension. In vitro, nutriosomes increased Caco-2 cell viability under oxidative insult (~120%) and demonstrated a protective effect against hydrogen peroxide-induced damage.

In vivo, Cannonau nutriosomes were orally administered to MPTP-treated mice. Despite the antioxidant potential demonstrated in vitro, immunohistochemical analyses of tyrosine hydroxylase and dopamine transporter in the caudate-putamen revealed no significant neuroprotective effects (Figure 1).

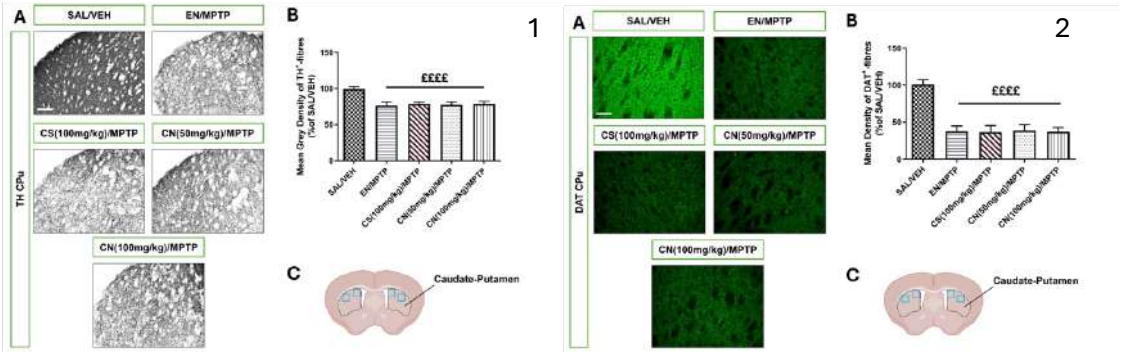

Figure 1: Effect of Cannonau suspension (CS) nutriosomes (CN) on the immunoreactivity of tyrosine hydroxylase positive (TH+)- fibres in the caudate-putamen (CPu) (1) and on the immunoreactivity of dopamine transporter (DAT)-positive fibres in the caudate-putamen (2) of MPTP-treated mice.

This was in contrast to previous data with Nasco nutriosomes, suggesting a critical role of procyanidin B2 or specific polyphenolic composition in mediating neuroprotection. The slower release and prolonged systemic availability of the Cannonau extract may support antioxidant effects but appears insufficient to counteract neurodegeneration in this model.

These findings highlight the importance of extract composition in designing neuroprotective formulations and suggest that combining targeted polyphenols with controlled-release systems may enhance therapeutic outcomes. Future work should focus on optimizing the polyphenol profile and investigating synergistic strategies to boost the efficacy of vesicular nutraceuticals in neurodegenerative disease models.

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Innovative Probiotic-Enriched Fermented Juices: Development and Functional Evaluation

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Keywords: Fermented juices, Probiotics, Metabolomics, Functional foods, Postbiotics

Functional fermented beverages have gained increasing attention due to their potential health benefits, particularly when enriched with probiotics. However, most commercially available probiotic drinks are dairy-based, limiting their accessibility for individuals with lactose intolerance or dairy allergies. [1]

This study investigated the development of novel functional fermented juices using a probiotic consortium of *Lactococcus lactis* subsp. *Hordniae* (LAB1), *Lactococcus lactis* subsp. *lactis* (LAB2), and *Pichia kudriavzevii* (Y1), isolated from homemade kefir. [2] Annurca apple peels, goji berries, and beetroots were chosen as substrates, known for their nutritional and functional properties, such as antioxidant content, vitamins, and bioactive compounds. The extracts were prepared via rapid solid-liquid dynamic extraction (RSLDE) using a Naviglio extractor, [3] a sustainable green technology that enhances extraction efficiency without thermal degradation.

Substrates were extracted with water, filtered, and inoculated with the probiotic consortium at concentration of 10^3 cells mL^{-1} (1:1:1 ratio). Fermentation was carried out at 37 °C for 72 h, monitoring microbial growth by plate colony counting, and pH change. The resulting fermented juices were evaluated for antioxidant activity (DPPH and ABTS assays, [2, 4]), antimicrobial properties against *Salmonella enterica*, *Staphylococcus aureus*, and *Listeria monocytogenes* (time-kill assay [5, 6]), and metabolic changes using GC-MS based metabolomics. Metabolites were extracted with ethyl acetate, derivatized, [7] and analyzed.

The results confirmed the viability of probiotic microorganisms in the substrates, with variable growth kinetics and a significant reduction in pH during fermentation. Fermentation led to significant increases in antioxidant and antimicrobial activities compared to unfermented controls, highlighting the functional benefits of these juices. Metabolomic analysis revealed distinct metabolic profiles post-fermentation, with PCA showing clear separation between fermented and unfermented samples. Supervised analysis identified upregulated metabolites, including phenolic acids, aryl-lactates, and hydroxyacids, which are indicative of beneficial postbiotics, such as 3-phenyllactic acid, [8, 9] tyrosol [10, 11] and 15-hydroxylinoleic acid. [12]

These findings highlight the potential of these new fermented juices as functional, dairy-free foods. Thanks to the naturally occurring bioactive compounds, they offer health benefits, making them a viable option for those seeking alternatives to dairy products.

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Environmentally friendly strategies to manage oromucosal oxidative stress-related pathologies: from grape processing by-products to polyphenol-rich buccal films

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Keywords: Buccal film; Pectin; Hyaluronic acid; Polyphenols; Waste recovery

Several oromucosal pathologies (e.g., oral mucositis, oral lichen planus, recurrent aphthous stomatitis) are strongly related to oxidative stress and could benefit from preventive/adjuvant treatments based on antioxidants administration. Polyphenols are a valuable solution possessing also anti-inflammatory, chemopreventive and wound healing effects. However, they result in low oral bioavailability, thus needing to be topically delivered by an ad hoc designed oromucosal formulation. For the specific purpose of treating oral lesions, buccal films represent the best option. They are soft and deformable formulations, designed to be mucoadhesive and useful for their wound dressing effect. Therefore, the aim of this work was to develop polymeric matrix buccal thin films containing polyphenols. To integrate this main objective with a current sustainability framework, polyphenols were not used as pure raw materials, yet they were recovered from a grape processing industry by-product: the waste black bentonite (BB). [1,2] The latter is a mixture of montmorillonite mineral clay and active carbon, used for removing protein haze from white musts/wines. Therefore, the 1st part of this work was focused on the development of an antioxidant polyphenol-rich secondary raw material obtained by green extractions from the waste BB. Both "wet" (due to the residual must content) and "dry" (subjected to freeze-drying) BB underwent 7 consecutive maceration cycles using PEG200 as an unconventional solvent, chosen for its established pharmaceutical and cosmetic applications. Extracts were characterized in terms of % recovery, density, pH (after dilution), total phenolic and protein content, gallic acid (GA), resveratrol (RSV) and quercetin (QRC) concentration, and antioxidant power. By integrating the obtained data, the 3rd extraction step from the wet BB (BentoPhen200 extract) was chosen for film formulation, achieving a % recovery exceeding 40% and a high antioxidant power (GAE ≈ 4 mg/g). Then, 2 buccal films were developed by solvent casting and compared: BentoPect (composed by BentoPhen200, Pectin and Calcium Lactate) and BentoPect-Hyalu (including Hyaluronic Acid). Both films appeared uniform, thin, soft and flexible, as well as resulted mucoadhesive and homogeneous in terms of weight, thickness and polyphenols content. The presence of the highly hygroscopic hyaluronic acid in the BentoPect-Hyalu formulation enhanced the swelling index and promoted the rate of matrix erosion thus resulting in a rapid dissolution respect to the BentoPect film. Finally, both films enhanced polyphenols accumulation into ex vivo porcine buccal tissues while avoiding actives permeation and absorption. Ultimately, the BentoPect formulation resulted superior so that it was able to produce a time-dependent tissue accumulation of GA, RSV and QRC obtaining, after 3 h of treatment, an entrapment of more than the 5, 23 and 7% respectively, of the starting polyphenols dose contained into the film.

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Environmental Pollution And Asthma: Exposomic Approach

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Keywords: HPLC-MS/MS, GC-MS, Pollutant, Biomarkers, Urinary.

Asthma is one of the most common respiratory diseases in the world, with an estimated 300 million asthmatic patients of all ages. Evidence suggests that air pollution has a negative impact on asthma outcomes in both adult and pediatric populations; to understand the role of pollution in the development of asthma it is necessary to evaluate and quantify biomarkers of dose and effect in the biological matrices of exposed subjects, as well as the role of polymorphisms of biotransformation enzymes as determinants of individual susceptibility. To study this, in healthy and asthmatic volunteers a spot urine sample and buccal brush sample were collected. Urinary levels of Benzene, Toluene, Ethylbenzene and Xylenes (BTEX), Styrene and Methyl-tert-butylether (MTBE) have been quantified to assess environmental exposure by GC-MS, the nucleic acids damage, as biomarker of effect, have been quantified by LC-MS/MS and HMGB1 have been determined in urine with enzyme-linked immunosorbent assay. [1] We recruited 450 subjects divided into healthy subjects (200 adults and 100 children) and asthmatic ones (100 adults and 50 children), living in Parma and occupationally unexposed to oxidant agents or environmental air pollutants. The LC analysis was done with ExcionLC (sciex), the separation was performed by C18 column in elution gradient, coupled by 6500+ (sciex) and the analysis was conducted in positive mode for metabolite and negative mode for oxidative nucleic acid; GC analysis was performed by SPME extraction to quantified low concertation of pollutant. In adults, urinary levels of most exposure biomarkers were statistically higher in asthmatic subjects than in healthy ones. The differences are preserved also by evaluating smoking habits and BMI. In children, we observed that asthmatic group excreted more biomarkers of exposure and of effects than healthy one even if the increase was not statistically significant. Positive correlations between DHBMA and 5MeCyt are observed, which are equal to 0.655 for healthy subjects ($p<0.01$) and 0.575 for asthmatic subjects ($p<0.01$). The correlation between inflammation and exposure could help in the personalized treatment of asthma, aiming to achieve a better quality of life for people with respiratory diseases [2]. In conclusion this study describes the difference between asthmatic and healthy subjects in oxidative stress levels, metabolites of toxic pollutants and the polymorphisms involved in their biotransformation in relation to the level of pollutants in the air of Parma and the result can be applied in the preventive field.

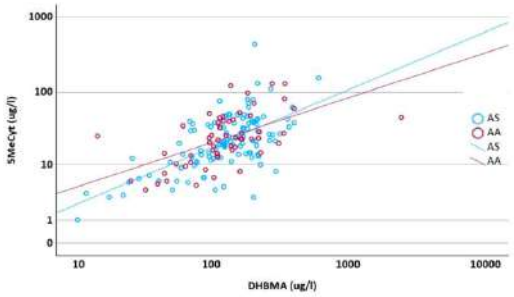

Figure 1: Correlation between DHBMA/5-MeCyt.

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Green Synthesis and Biological Evaluation of 1,2,3-triazoles

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Keywords: cyrene; green chemistry; 1,2,3-triazoles; synthesis; α-glucosidase.

1,2,3-Triazoles are considered privileged scaffolds due to their diverse biological activities, generating significant interest in the development of efficient and rapid synthetic methodologies. [1] Among these, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction stands out as the most effective and widely applied approach in click chemistry for the synthesis of complex structures *via* a triazole linker. [2] Solvents play a crucial role in CuAAC by solubilizing the reaction components, namely azides, alkynes and the Cu(I) catalyst, which is generated *in situ* from a Cu(II) salt and a reducing agent. Commonly used solvents such as dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF) and tetrahydrofuran (THF), however, have significant drawbacks: DMSO and DMF are classified as reprotoxic (H360), while THF is highly flammable and prone to hazardous peroxide formation. [3]
Herein, we report the first sustainable protocol utilizing Cyrene, a biodegradable and non-toxic solvent, for the synthesis of 1,2,3-triazoles (Figure 1). [4] This method circumvents the use of toxic solvents and offers significant operational advantages, such as product isolation through simple precipitation in water, eliminating organic solvent extractions and chromatographic steps. Consequently, this approach not only reduces waste generation but also lowers operational costs, making it feasible to conduct the reaction on a gram scale. Its broad applicability is demonstrated with complex substrates, such as biologically active coumarins and triazole-linked bifunctional molecules. Additionally, this protocol allows a one-pot, three-component reaction with organic halides, terminal alkynes, and sodium azide, avoiding the need to isolate organic azides, which are unstable and environmentally sensitive intermediates. This sustainable approach establishes a versatile and efficient pathway for triazole synthesis, aligning with the principles of green chemistry.
The synthesized triazole compounds exhibited notable biological activity, particularly as potent inhibitors of α-glucosidase and α-amylase enzymes. Enzymatic assays showed a high inhibition rate (>90%), with IC_{50} values lower than those of the reference drug, Acarbose, highlighting their promising therapeutic potential for metabolic disorders related to carbohydrate metabolism.

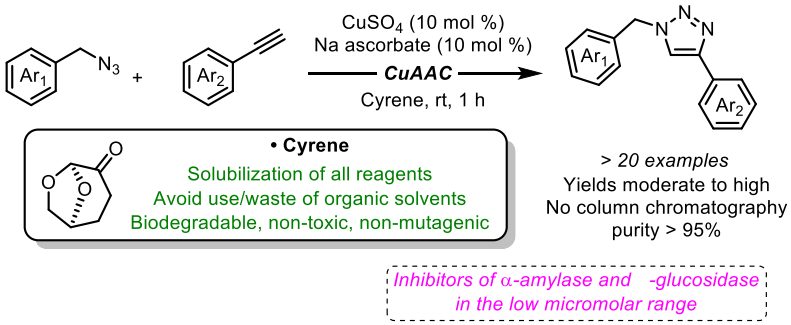

Figure 1: 1,2,3-triazole synthesis in the green solvent Cyrene.

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The Different Effects of Two Global Warming Conditions on Two Sea Urchin Species Exposed To Environmental Pollutants

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Keywords: Climate change, Pollution, Echinoderms, Sea urchin embryo, Environmental monitoring

Gradual ocean warming and marine heatwaves represent major threats for marine organisms already facing other anthropogenic-derived hazards, such as chemical contamination in coastal areas. Sea urchin larvae are a common model system in marine ecotoxicological studies to assess the impacts of climate change and pollution. Here I report the combined effects of thermal stress and exposure to three different pollutants of the marine environment (gadolinium, vanadium and phthalates) on embryos of two key Mediterranean Sea urchin species with predicted opposite responses to global warming, the temperate *Paracentrotus lividus* and the sub-tropical *Arbacia lixula*. These species are among the most abundant echinoids living in shallow rocky reefs of the southwestern Mediterranean and are ecologically important because their herbivorous grazing impacts macroalgae and leads to the formation of barrens habitats. Embryos were exposed to combined treatments of three temperatures (18°C, 21°C, 24°C) and a wide range of concentrations of the three pollutants (from environmentally relevant to cytotoxic). We tested the single and combined effects to thermal stress and pollutants at two functional levels: i) exposure–response relationships, analyzing the effects in terms of abnormal development and mortality; ii) morphological, analyzing impacts of treatments on larval phenotypes and morphometric traits of larval growth and biomineralization. With respect to developmental progression, elevated temperatures at near-future projections (+3°C, 21°C) accelerated development and achievement of the larval stage, while extreme warming at present-day marine heatwave conditions (+6°C, 24°C) breached the thermotolerance threshold of both species with a higher proportion of abnormal larvae.

We found a fascinating double side effect of increased temperature combined to pollution: a mild temperature increase (+3°C) reduced the negative effects of pollutants on development with a lower percentage of abnormality and improved skeleton growth, while combined heatwave conditions (+6°C) and pollution resulted in a lower proportion of embryos reaching the advanced larval stages.

Our results indicate that the negative effects of pollutants-exposure on *P. lividus* and *A. lixula* larval development and biomineralization will be mitigated by near-future ocean warming temperatures, up to a thermotolerance threshold triggering negative synergistic effects. Our data highlight the need for a better understanding of the interactions between the multiple stressors faced by marine species in coastal environments.

Decorated nanogels for modulating glial cells in spinal cord injury

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Keywords: spinal cord injury, nanogel, drug delivery, expansion microscopy, selectivity

Spinal cord injury (SCI) is an invalidating disease that involves the damage of the spinal cord or the nerves connecting the spine to the central and peripheral nervous system. [1] This pathology is characterized by the primary SCI, that is the consequence of the traumatic event, and by the subsequent inflammatory response, characterized by the activation of microglia/macrophages/astrocytes, that leads to an aggravation of the pathology and to neurodegeneration. [2] A possible therapeutic approach is represented by the possibility to modulate the inflammatory response through the release of drugs in the damaged zone selectively within different cell lines. Recent advances in polymer science and nanotechnologies showed an increased interest for the nanogels (NGs), a new class of colloidal systems that, if properly functionalized, can be used as carriers of drugs to treat SCI. Nanogels were synthesized using polyethylene glycol (PEG) and linear polyethylenimine (PEI). [3] This PEI functionalization is essential for being able to constantly trace the nanogels during the biological assays. The so produced NGs have been coated in order to give to the material selectivity towards specific cells. The degree of selectivity and subcellular distribution of the NGs have been evaluated through expansion microscopy, an innovative and super-resolution microscopy technique. Different administration schemes have been tested in a SCI mouse model, namely an early and a late administration, showing that a significant improvement of the locomotor performances can be obtained if the NG is injected immediately after the injury (Figure 1). The nanovectors have been demonstrated to be highly selective and functional in the treatment of the glial component in different phases of SCI, paving the way to clinical applications. [4]

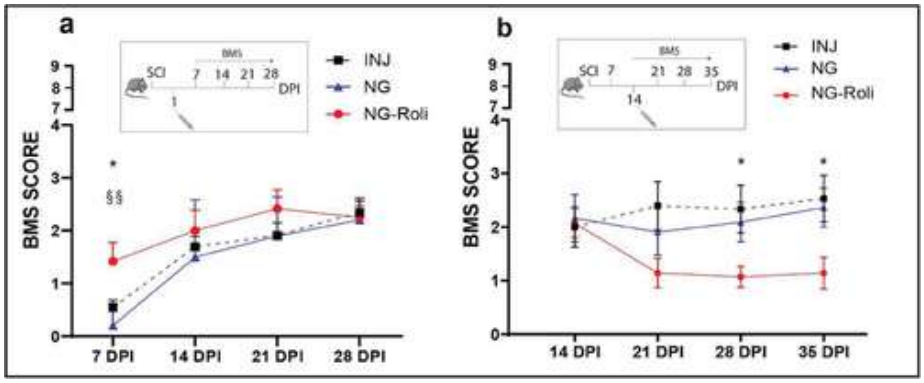

Figure 1: a) Injection of NG loaded with Rolipram in SCI mice on 1 DPI resulted in a significant improvement of locomotor performance compared to the untreated (INJ) or NG-only (NG) groups examined on 7 DPI. B) Injection of NG loaded with Rolipram in SCI mice on 14 DPI resulted in significant impairment of locomotor performance compared to the INJ or NG group examined at 28 and 35 DPI.

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Evaluating Astaxanthin-Loaded Nanoparticles as Drug Delivery Systems for Ocular Diseases: an LC-MS/MS-Based Investigation

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Keywords: Astaxanthin, biodegradable-nanoparticles, Orbitrap mass spectrometry

Astaxanthin (AST), a powerful natural antioxidant, holds promise for ocular disease treatment, but its clinical application is compromised by instability. [1] To enhance its bioavailability, AST-loaded nanoparticles (NPs [AST]) have been developed and optimized. [2,3] Our collaborators have optimized NPs $[AST]^+$ to enhance permeability and bioavailability, hypothesizing their potential as a therapy for ocular diseases. This project aims to characterize the newly synthesized NPs $[AST]^+$ and investigate their molecular effects in retinal cells by LC-MS/MS. AST was extracted from *Haematococcus pluvialis*, encapsulated in biodegradable NPs, and surface-derivatized to enhance permeability. Physicochemical characterization was performed by dynamic light scattering (DLS) experiments using the Nano ZetaSizer ZS instrument. Cell viability in ARPE-19 cells exposed to hydrogen peroxide (H_2O_2), with or without treatments (AST, NPs, NPs $[AST]^+$), was assessed by spectrophotometry. A transwell migration assay evaluated the uptake of NPs $[AST]^+$ by retinal cells after 6h and 24h via confocal microscopy. To elucidate the molecular mechanisms underlying the effects of NPs $[AST]^+$, an LC-MS/MS-based proteomics experiment was conducted. Retina cells were treated with AST, NPs, or NPs $[AST]^+$, with or without H_2O_2. Cells were harvested after 6 and 24 hours for protein extraction. Peptides were analyzed using an Ultimate 3000 nano-UHPLC system coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer. MS1 spectra were acquired in data-dependent acquisition (DDA) mode using the Orbitrap analyzer (m/z range of 375–1500, resolution 120,000), while MS2 spectra were acquired at resolution of 50,000. Protein identification and label-free quantification (LFQ) were performed using Proteome Discoverer (v2.5), applying a Student's t-test on normalized protein intensities. Proteins with $p < 0.05$ and fold change (FC) ≥ ±1.3 were considered significantly differentially expressed (DEPs). Functional enrichment analysis was conducted using Cytoscape (v.3.10.1) and protein-protein interactions analysis by STRING (v.12.0). DLS results showed AST loading efficiency in NPs ranging between 37.3% ± 2.4 and 80.9% ± 6.4. Viability assays confirmed that empty and NPs $[AST]^+$ were non-toxic, maintaining viability > 80% after 24h. Interestingly, NPs $[AST]^+$ preserved ARPE-19 cell vitality above 90% under oxidative stress. The transwell migration assay confirmed NPs $[AST]^+$ uptake at 6h and 24h, with confocal microscopy showing significant NPs migration and cellular uptake. LFQ proteomics identified 40, 60, and 63 modulated proteins in AST, NPs [AST]+, and NPs vs control (CTRL), with 68 DEPs in AST vs. NPs $[AST]^+$. After 6h H_2O_2 exposure, 23, 44, 36, and 49 DEPs were found in the same comparisons. Cytoscape analysis highlighted enriched pathways, with NPs $[AST]^+$ down-regulating proteins linked to apoptosis execution and lipid biosynthesis while up-regulating those involved in steroid biosynthesis and eye development under oxidative conditions compared to empty NPs. This pioneering LC-MS/MS-based investigation on AST-loaded nanoparticles in retinal cells suggests that NPs $[AST]^+$ are promising carriers for ocular drug delivery, warranting further clinical exploration.

Acknowledgements

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Gelatin-Based Double Network Cryogels: Influence of Gelatin Properties on Mechanical and Biological Performance in 3D Cell Culture

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Keywords: double-network cryogels, gelatin methacryloyl, 3D cell culture, mechanical properties, sol-gel transition temperature

3D cell culture refers to the technique of growing cells within a three-dimensional environment, which more accurately mimics the natural architecture and physiological functions of tissues in the human body. 3D systems promote more realistic cell–cell and cell–matrix interactions, making them valuable for drug screening and tissue engineering. Among the most common 3D culture approaches are scaffold-based systems, which utilize a 3D support that enables cells to adhere, proliferate and organize in a way that closely resembles in vivo tissue behaviour. In this context, double network cryogels (DN) are emerging as highly promising scaffolding materials for 3D cell culture. [1] DN are a particular type of cryogels consisting of 2 interpenetrating and independently crosslinked polymer networks. This dual-network design imparts enhanced mechanical strength, while preserving the high interconnected macroporous structure and the sponge-like elasticity typical of cryogels.

Among the various biomaterials employed in scaffold fabrication, the methacryloyl derivative of gelatin (GelMA) is widely used for the design of single- and double-network cryogels. [2] This is primarily due to its excellent biocompatibility and its intrinsic ability to support cell adhesion, proliferation and differentiation. Despite its widespread use, the influence of GelMA physico-chemical properties on the mechanical behaviour and biological performance of DN cryogels, remains an area that is not yet thoroughly explored. GelMA-based DNs are fabricated using the cryogelation technique, a radical crosslinking process that occurs at subzero temperatures. Under these conditions, the sol–gel transition temperature (T_g) of GelMA can significantly influence the outcome of the cryogelation process. Indeed, the simultaneous occurrence of both physical and chemical crosslinking may impact the architectural and mechanical properties of the resulting DNs, which play a critical role on the biological performance of the scaffold. Therefore, the aim of this research was to investigate how variations in the rheological properties of GelMA, and in particular its T_g, influence the mechanical characteristics and, in turn, the biological functionality of the resulting DN cryogels. To this end, GelMA derivatives with different T_g values were considered, which were obtained by using gelatin from different sources (porcine and fish) or through specific synthetic procedures designed to modify the T_g of GelMA. [3] All types of GelMA were first characterized for triple-helix content and rheological properties, then they were employed to prepare single and double-network cryogels. The morphology and mechanical properties of the prepared scaffolds were investigated by SEM analysis and mechanical compression tests, and correlated with the ability to support cell viability and proliferation. Overall, these findings can aid in the design of efficient GelMA-based scaffolds with broad applications in 3D cell culture.

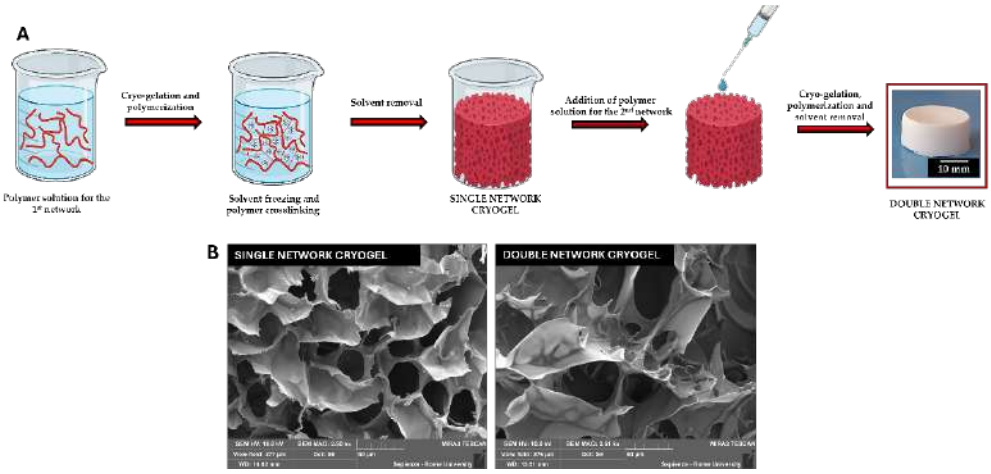

Figure 1: (A) Single- and double-network cryogel formation procedure and (B) SEM images.

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Groundbreaking NanoMIP technology for adenosine detection and integration in antibody-free bioassays

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Keywords: Molecular Imprinted Polymer, NanoMIP, Adenosine, bioassay

Traditionally, in the field of immunodiagnostic, antibodies, receptors, or natural enzymes have been considered the gold standard for molecular recognition. [1] However, the drawbacks associated with antibody production have driven the exploration of alternative molecular recognition materials systems. A practical alternative can be found in nano-molecularly imprinted polymers (nanoMIPs), which offer high stability, cost-effectiveness, and adaptability to different environmental conditions. These characteristics make them suitable for bioanalytical applications (e.g.: diagnostics). [2] One notable biological target is adenosine, a nucleoside that plays an essential role in cardiovascular risk, stress response, and metabolic dysfunctions, making its monitoring essential. In the specific context of the molecular imprinting technique, adenosine has proven to be a particularly challenging template due to its chemical properties. This difficulty arises from solid-phase polymerization synthesis (SPPS), [3] used to produce nanoMIPs, which require the immobilization of the template on the nanobead's surface during the imprinting step. Different adenosine anchoring strategies were done to overcome these difficulties to obtain an imprinted polymer. The objective was maintaining the purine base (adenine) exposed, thereby maximizing the imprinting effect. The ribose moiety was modified with various functional groups, including amine, carboxyl, aldehyde, and succinate, or was directly linked via periodate oxidation to the aminated solid phase. The polymerization mixture consisted of acrylic acid, N-isopropyl acrylamide, tert-butylacrylamide, methylen-bis-acrylamide, and a modified thymine as the complementary base of adenosine. This approach enables the development of nanoMIP with a binding affinity of about $3.6 \times 10^5\ M^{-1}$. Beyond this achievement, an even more ambitious goal is to integrate nanoMIP, as the molecular recognition element, in the development of an ELISA-like assay for the adenosine detection, offering a synthetic alternative to traditional antibodies. The bioassay was designed in the competitive format: ELISA microplates were first coated with bovine serum albumin (BSA), then nanoMIPs were grafted to amine function by DIC/NHS method. Different polyadenine-labeled biotin (polyA-b) were used to compete with free adenosine for the nanoMIP binding site. Detection was carried out using streptavidin-HRP as the signal reporter enabling the enzymatic colorimetric reaction. The synthesis of the nanoMIP and the functioning scheme of the ELISA-like nanoMIP-based bioassay was shown in Figure 1. This approach allows for integrating nanoMIPs in the conventional ELISA platform as the molecular recognition element, enabling the realization of an antibody-free assay. Replacing antibodies with synthetic alternatives has always been an ambitious target for molecular imprinting, and this work represents a remarkable step forward in this challenge.

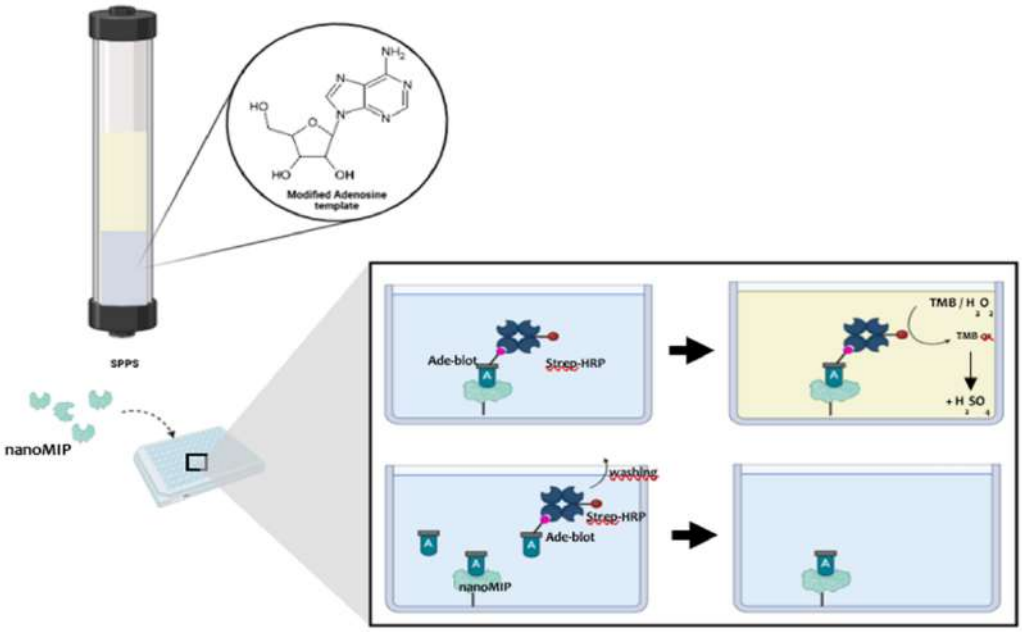

Figure 1: Schematic design of nanoMIP synthesis and integration to bioassay.

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Tuning the Dual HDAC6/Hsp90 Inhibition by Purine-Based Hydroxamic Acids

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Keywords: Drug Discovery, HDAC6, Hsp90, Prostate Cancer, Multitarget Approach.

The ability of a single molecule to hit different biological targets is certainly an appealing strategy in drug discovery, especially for the treatment of complex diseases, such as cancer. HDAC6 and Hsp90 are well-known enzymes which are involved in essential cellular processes such as growing, proliferation, migration and apoptosis. [1] These enzymes have gained significant attention for the development of new antiproliferative agents, while blocking the activity of both enzymes with dual inhibitors offers an attractive multitarget approach. [2] Furthermore, the interplay between HDAC6 and Hsp90 suggests that concurrent inhibition of both enzymes can synergistically enhance antitumor effects towards different cancer cell lines. [3,4] Herein we investigated the design, synthesis and biological assessment of a new class of purine-based HDAC6/Hsp90 by combining the structural requirements of HDAC6, along with the exploration of two distinct Hsp90 binding pockets, the "inner" and "outer" subpockets (fig.1). The initial screening identified compound **17** as lead compound, endowed with high potency and selectivity against both HDAC6 and HSP90. This compound has been selected for further biological evaluations, exhibiting strong antiproliferative activity across three prostate cancer cell lines of varying aggressiveness. The cellular engagement of the two biological targets has been confirmed as well as the potential antiproliferative activity on prostate cancer-related organoids, a 3D model which simulates the *in vivo* architecture of tumors. These studies, in combination with *the in vitro* drug-like analysis, pointed out these multitarget compounds as promising agents for further preclinical evaluation against aggressive prostate cancer.

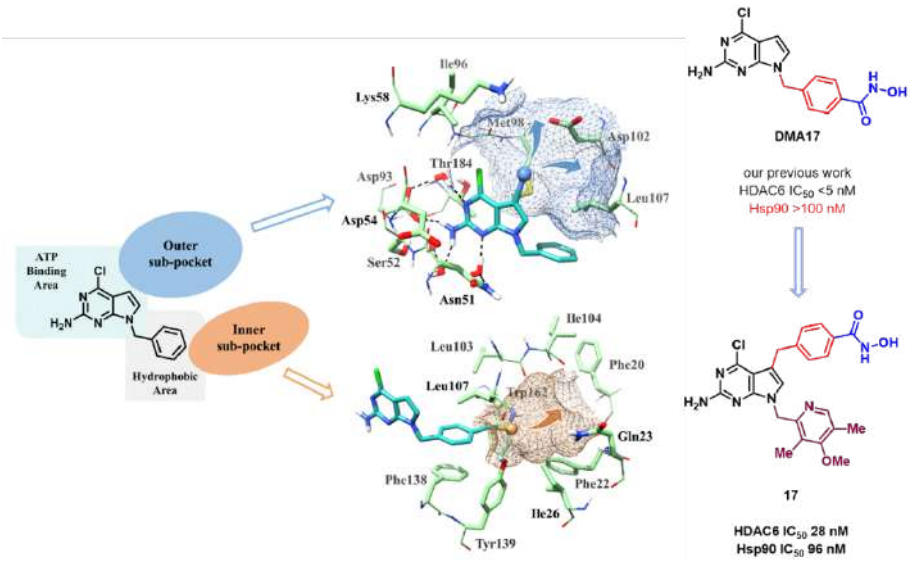

Figure 1: Rational design of dual HDAC6/Hsp90 inhibitors exploiting the two Hsp90 subpockets.

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Deficiency in NPC2, results in disruption of mitochondria-late endosome/lysosomes contact sites and endo-lysosomal lipid dys-homeostasis

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Keywords: Niemann–Pick-C Type 2, Cholesterol , glucosylsphingosine, mitochondria-lysosomes membrane-contact-sites

Dysfunction of the endo-lysosomal intracellular Cholesterol transporter 2 protein (NPC2) leads to the onset of Niemann–Pick Disease Type C (NPC), a lysosomal storage disorder. [1]
Metabolic and homeostatic mechanisms are disrupted in lysosomal storage disorders (LSDs) [2] hence we characterized a cellular model of NPC2 gene knock out, the NPC2−/− HEK cell line, to assess alterations in organellar function and inter-organellar crosstalk between mitochondria and lysosomes. We performed characterization of lipid alterations and confirmed altered lysosomal morphology, but no overt changes in oxidative stress markers. Using several techniques, we demonstrated that contacts between mitochondria and late endosomes/lysosomes are reduced in NPC2−/− HEK cells, we observed that the acidic compartments are swollen and lipid dense. Quantification of endogenous lipids in HEK$^{NPC2-/-}$ cells by mass spectrometry reveals accumulation of lipid species indicative of sphingolipid metabolic dysregulation within the lysosome. Specifically, HEK $^{NPC2-/-}$ cells exhibit marked elevation of glucosylsphingosine and glucosylceramides, substrates of beta glucocerebroside (GBA), as well as accumulation of sphingosine and sphingomyelins. [3] Our studies suggest an involvement of NPC2 in the formation of contact sites between mitochondria and lysosomes and support the hypothesis of a role for NPC2 in the endo-lysosomal trafficking pathway and dynamic organellar crosstalk.

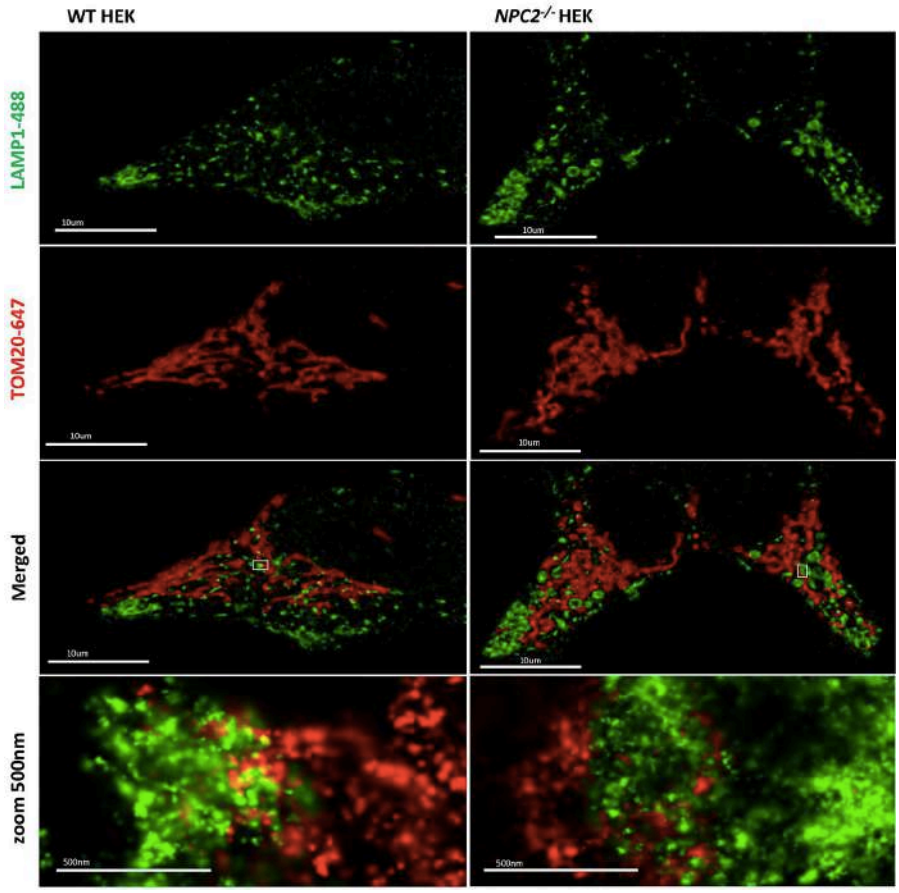

Figure 1: Morphology of late endosomes/lysosomes in WT and NPC2−/− HEK cell lines. a dSTORM (direct Stochastic Optical Reconstruction Microscopy) images of 4% PFA fixed WT and NPC2−/− HEK cells co-stained with antibodies against endogenous TOM20 (red; outer mitochondrial membrane) and LAMP1 (green; lysosomal membrane). Images were acquired with the ONI microscope. For each cell line, WT HEK and NPC2−/− HEK, the mitochondrial and late endosomal/lysosomal networks are represented. Scale bar = 10 µm. The insets (bottom row) show contact sites between the lysosomal and the outer mitochondrial membranes with more overlap in the WT cells. Scale bar = 500 nm.

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Structure-Activity Relationship Study of Quaternary Ammonium Salts: Synthesis, Characterization, and Antibacterial Evaluation

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Keywords: quaternary ammonium salts (QASs), ionic-liquids (ILs), antibacterial agents, antifouling

The spread of multidrug-resistant bacterial strains poses a serious threat to public health, prompting the search for new antimicrobial agents to address biofilm-associated infections. [1] Biofilms, which are notoriously difficult to treat, feature a protective exopolysaccharide matrix embedding bacterial cells, enhancing their resistance to conventional antimicrobial treatments. [2] In addition to the health risks, biofilms cause economic damage in industries such as marine, construction, and food, where they contaminate equipment. [3] Quaternary ammonium salts (QASs), including those with ionic liquid properties, are promising antibiofilm agents. [4]
This study outlines the synthesis of new structurally-optimized antibacterial QASs, some of which possess ionic liquid properties, evaluating their effectiveness against the gram-negative *Pseudomonas delhiensis* PS27 strain. This strain, isolated from soil and groundwater samples contaminated with hazardous perfluoroalkyl and polyfluoroalkyl substances (PFASs), is remarkably resistant to environmental stresses. [5] Understanding the thermophysical properties and liquid operating temperature range of these compounds was critical for evaluating their practical applications. To this end, the synthesized salts were characterized using differential scanning calorimetry (DSC) to study phase transitions and thermogravimetric analysis (TGA) to assess thermal stability. Furthermore, the possibility of incorporating the most promising salts into polymeric matrices or oily bases was explored for the development of antibiofilm materials and slippery-liquid-infused porous surfaces (SLIPs).

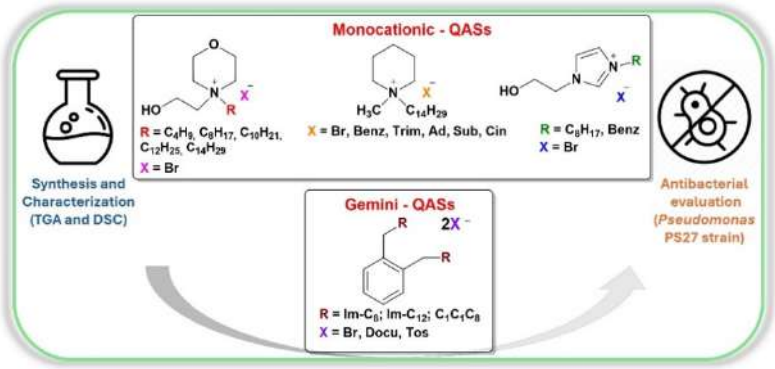

Figure 1: Synthetized Quaternary Ammonium Salts (QASs) evaluated as new antibacterial agents.

Acknowledgments

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ORAL SLAMS

An aptamer-based rapid diagnostic test for the simultaneous detection of malaria and dengue

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Keywords: dengue, malaria, rapid test, lateral flow assay

The resurgence of international travel after the COVID-19 pandemic has significantly increased the incidence of travel-related infectious diseases, particularly malaria and dengue. In 2023, over 1 billion people traveled internationally, with nearly 18% experiencing fever upon return. Among those arriving from endemic regions, nearly half were diagnosed with one of these high-morbidity diseases. [1] Since a number of international travelers come from non-endemic regions, they are especially vulnerable due to their lack of prior immunity. Diagnosing malaria and dengue in these areas is challenging, as microscopy and PCR are costly, time-consuming, and require specialized labs. Without rapid and accessible diagnostic tools, treatment is often delayed, increasing the risk of severe complications. In consequence, a rapid diagnostic tool for both diseases would enable the point-of-care testing, improving patient triage, lowering healthcare costs, and mitigating disease spread by preventing autochthonous outbreaks in regions experiencing climate-driven expansion of mosquito vectors. In this context, we propose a novel lateral flow assay (LFA) capable of simultaneously detecting malaria and dengue, especially tailored for international travelers. Unlike existing LFAs, which either focus on a single disease or show poor analytical performance, our approach utilizes aptamers—short synthetic DNA or RNA sequences that bind to target molecules with high specificity. Compared to traditional antibody-based detection, aptamers offer superior specificity, greater stability, and lower production costs, making them ideal for a next-generation rapid diagnostic test. [2]

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Mitigation of environmental toxicity in perovskite solar panels through perfluoroalkylated additives

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Keywords: Photovoltaic, toxicology, metal, leaching, voltammetry.

Most of the energy today is produced from non-renewable energy sources (fossil fuels such as coal, oil and natural gas, etc.), whose combustion generates greenhouse gases (CO_2, CH_4, N_2O, etc.),leading to an increase in global temperature[1]. For this reason, new alternative sources of energy have been studied, among which photovoltaics is currently one of the most prominent, given the almost infinite availability of energy and the easy access to the source. For decades, the photovoltaic market has been dominated by silicon cells, despite presenting several issues. In the last twenty years, the attention of researchers has shifted to perovskite solar cells. Since their first applications in 2009[2], these cells are characterized by excellent characteristics such as high efficiency and lightness. However, the bottleneck of this technology is related to the presence of lead in the photoactive layer. Lead is known to cause various ailments to humans, such as insomnia, fatigue, heart problems and weight loss, being classified as probably carcinogenic to humans (Group 2) in 2006 (IARC, 2006) [3]. The biggest problem occurs when cells are disposed of at the end of their life or in case of accidental breakages caused by meteorological or human phenomena. The toxicity of Pb is ranked 2nd in the priority list of toxic substances, respectively (ATSDR, 2015). Currently, there is no adequate treatment for photovoltaic devices that contain hazardous metals and their recovery [4] and, highlighting the importance to quantify metals of toxicological interest resulting from the leaching of waste materials into environmental matrices. For this purpose, leaching tests can be used to investigate the role of different organic or inorganic molecules used to reduce the release of toxic elements into the environment. Herein, we show how simple voltammetric techniques (rapid, cheap and precise) [5] can rapidly and accurately quantify the release of lead ions in aqueous solutions from these perovskite based solar cells. In addition the use of a perfluoroalkylated pyrene additive added to the hole transporting material has been evaluated to establish its effect as a screening agent in the release of this metal. The results obtained are promising, the effect that these compounds have on improving the morphology of these systems has also been evaluated through atomic force microscopy techniques.

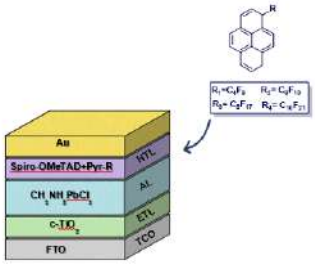

Figure: Schematic illustration of a perovskite solar cell with pyrene as an HTL additive.

Acknowledgments

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A fast separation method for coumarin and furocoumarins quantification in perfumes by means of Supercritical Fluid Chromatography

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Keywords: coumarin, fragrances, furocoumarins, green analytical chemistry, supercritical fluid chromatography.

Furocoumarins and coumarin are key fragrance components used in cosmetics, especially in perfumery. Notwithstanding their contribution to the olfactory profile of perfumes, the presence of these compounds gives rise to health concerns in view of their potential adverse effects. Furocoumarins have shown phototoxic properties, [1] causing cutaneous irritation upon UV exposure. On the other hand, coumarin has been associated with cases of skin sensitisation [2] and allergic reactions in certain individuals. In light of the aforementioned findings, the European Parliament and the International Fragrance Association (IFRA) have set maximum limits for these compounds in cosmetics, aiming to protect consumer safety. [3,4] In order to check compliance with these regulations, accurate and efficient analytical methods are needed for the detection and quantification of these substances in perfume formulations. Conventional analytical techniques such as high-performance liquid chromatography (HPLC) are often time-consuming and involve the use of toxic and environmentally harmful solvents as mobile phases. The present study proposes a faster and sustainable method for the determination of regulated coumarin and furocoumarins in perfumes using supercritical fluid chromatography (SFC) with photodiode array detection. Different stationary phases (HILIC, F5, C18, and C8), co-solvents and additives were tested for method development. Furthermore, a comprehensive investigation was carried out into various parameters (backpressure, modifier percentage, flow rate, and temperature) affecting the separation. The method was validated in terms of linearity, precision, accuracy, and limits of detection and quantification, demonstrating its suitability for routine quality control applications. Advantages of this approach over traditional HPLC methods consisted in: i) higher throughput (17 samples per hour), ii) low solvent consumption and waste (< 1 mL per analysis), iii) use of greener solvents and iv) reduced operational costs. Such features make the proposed method a promising tool for the fragrance industry, enabling for faster and more sustainable routine quality control.

Acknowledgments
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Synthesis, *in vitro* exploration, and reactivity studies of 6-chloropyridin-2-yl benzoates as promising covalent fragments with antiviral and pro-apoptotic activities

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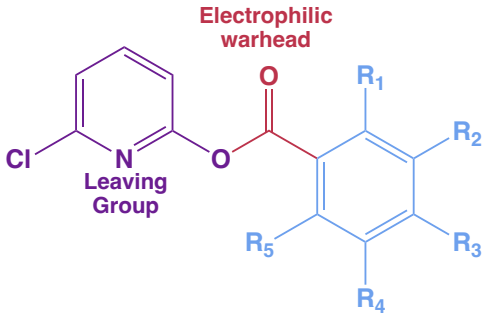
Keywords: ester, covalent inhibitors, antiviral activity, pro-apoptotic activity

Fragment-Based Drug Discovery (FBDD) focuses on identifying small molecular fragments that, despite their low molecular weight, can specifically bind to biological targets. [1-3] Due to their small size, ester-based inhibitors are particularly suitable as starting building blocks in covalent FBDD, aiding the development of potential bioactive compounds. [4] In this study, we synthesized a series of 6-chloropyridinyl ester compounds (Figure 1), which were screened for their dual anticancer and antiviral activity. These inhibitors take advantage of the electrophilic nature of the 6-chloropyridinyl ester warhead to form a covalent bond with reactive amino acid residues present in the targeted proteins. The 6-chloropyridinyl ester derivatives were first analyzed for their ADME properties and drug-likeness using the SwissADME tool [5] and then synthesized following two different one-pot procedures.



1a R_1=NO_2; R_4=Cl; R_2=R_3=R_5=H
1b R_1=NH_2; R_2=Cl; R_4 = Cl; R_3=R_5=H
1c R_1=R_5=H; R_2=R_3=R_4=OCH_3
1d R_3=OCH_3; R_1=R_2=R_4=R_5=H
1e R_3=CF_3; R_1=R_2=R_4=R_5=H
1f R_3=CH_3; R_1=R_2=R_4=R_5=H

1g R_1=R_2=R_3=R_4=R_5=H
1h R_3=$N(CH_3)_2$; R_1=R_2=R_4=R_5=H
1i R_3=F; R_1=R_2=R_4=R_5 =H
1j R_3=Br; R_1=R_2=R_4=R_5=H
1k R_1=R_2=Cl; R_3=R_4=R_5=H
1l R_3=Cl; R_1=R_2=R_4=R_5=H
1m R_2=Cl; R_3=F; R_1=R_4=R_5=H

Figure 1: Synthetized 6-chloropyridinyl esters 1a-m.

The ability of the compounds to form covalent bonds with reactive nucleophilic residues was confirmed by HPLC-MS studies. Therefore, the antiviral effect of 6-chloropyridinyl esters was evaluated through enzymatic inhibition assays, proving a binding affinity in the micromolar range. In particular, compound 1g exhibited the lowest IC_{50} value of 30.0 ± 6.6 μM. The antiproliferative activity was investigated in several tumor cell lines (A-375, MCF-7, and HCT-116) and normal cells (16HBE). The ester derivatives showed remarkable selectivity against the melanoma cell line A-375, with IC_{50} values of 1.59 ± 0.03 μM and 1.08 ± 0.01 μM for compounds 1e and 1m, respectively. Furthermore, these two compounds were analyzed for their ability to induce cellular cycle arrest, apoptosis, evaluation of mitochondrial membrane potential, and ROS-production. These results emphasize the potential of the 6-chloropyridinyl ester derivatives as promising covalent reactive fragments for both antiviral and anticancer applications.

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Stimuli-responsive hydrogels from low-complexity peptide domains

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Keywords: phase separation, peptides, supramolecular chemistry, stimuli-responsive hydrogels.

Defining soft biomaterials, such as stimuli-responsive hydrogels, is essential for advancing applications such as in targeted drug delivery, biosensing, and tissue engineering due to their ability to dynamically respond to environmental triggers. [1,2] In this study, we characterized phase-separating peptides and elucidated the principles governing their self-assembly into hydrogels. Low-complexity aromatic-rich kinked segments (LARKS) were employed as building blocks to generate stimuli-responsive materials. These domains, recognized, among the others, in hnRNPA1, hnRNPA2, and FUS proteins, and containing glycine, serine and tyrosine residues, are known to give rise to less tight association of the β-sheets inside the assemblies compared to steric zipper detected in amyloids, making the aggregates sensible to external stimuli. [3] By analyzing the properties of various multi-LARKS peptides, we developed a model informing the rational design of point mutations to modulate the mechanical properties and temperature stability of LARKS-based hydrogels, resulting in new stimuli-responsive matrices. Our findings were further supported by demonstrating that these hydrogels function effectively as drug-reservoir matrices capable of the efficient release of a model drug at 40°C, underlying their potential for biotechnological and medical applications as carriers for small molecules or nucleic acids.

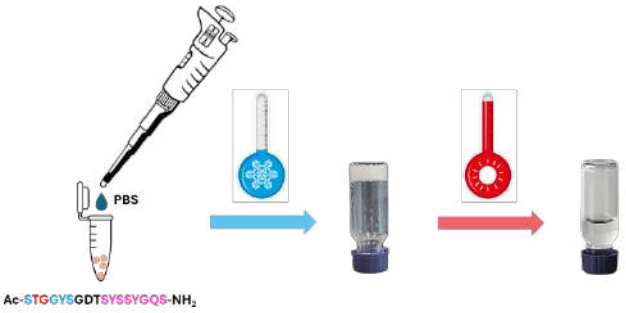

Figure 1: Schematic representation of hydrogel formulation and thermo-responsivity.

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Synthesis and SAR of 1,2,4-oxadiazole derivates as potential antibacterial agents

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Keywords: heterocycle, oxadiazole, antibacterial

Antibacterial resistance is increasing globally, making standard therapies for bacterial infections ineffective. [1,2] For this purpose, taking inspiration from a previously published study, [3] a series of novel 1,2,4-oxadiazole pyridinium salts derivates have been synthesized. Maintaining the central core, the bioactivity of different substituents in position 3 or 5 of oxadiazole have been explored, varying from small-length alkyl chains to variously substituted aromatic rings. The resulting 34 derivates have been tested *in vitro* against Gram-negative (*K.pneumoniae* and *E.coli*) and Gram-positive (*S.aureus, S.haemolitycus* and *E.faecium*) resistant strains with dilution and minimal inhibition concentration (MIC) methods. Through this structure-activity relationship (SAR) study, it was possible to demonstrate that as the substituent on the aromatic ring changes, the activity of the antibacterial compounds also changes. For example, an electron donating group ($-OCH_3$) on the phenyl ring, compared with an electron withdrawing group ($-NO_2$ and -F), causes a reduction in MIC values. On the other hand, if the substituent is an amide, the antibacterial activity of the compounds disappears. While aliphatic analogues did not show any activity against the resistant strains.

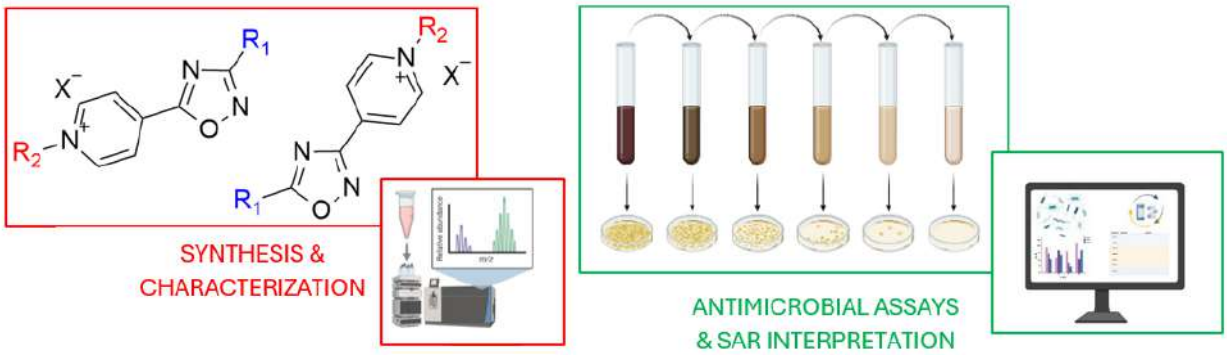

Figure 1: Workflow representation.

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Newest approach for micro- extraction from complex matrix.

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Keywords: Fabric Phase Sorptive Extraction, Green Chemistry, Device, Micro-extraction, Sample treatment.

Until Green Chemistry, Green Analytical Chemistry and Green Sample Preparation were born, scientists focused their research on minimizing waste, reducing sample treatment, volume and solvent used. For this reason, in 2014 Kabir and Furton patented a new micro-extraction method, called Fabric Phase Sorptive Extraction (FPSE). [1] This approach permits to avoid significantly sample treatment and the FPSE could directly put into the sample and analytes are adsorbed on the phase. In multi- analytes methods and due to chemistry of FPSE, sometimes the extraction is not the best for each compound. Based on this, Kabir and colleagues patented in 2024 a device which permits to extract simultaneously different analytes using more FPSE, by leveraging various surface chemistry of the sol- gel. [2] The device has permitted to improve extraction yield of many compounds with different chemical characteristics in complex matrix, as plasma, blood, urine, and saliva could be. The device allows the ease extraction in biological and environmental matrices, the configuration permits to use a magnetic stirrer, increasing permeability of FPSE. Its size and configuration make it suitable for small volume. The configuration permits to add up to six different surface chemistry FPSE, in this way recovery will be maximized. The device has shown good results demonstrating applicability in many fields.

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Lead optimization of specific OPA1 inhibitors enhancing cytochrome c release and cell death

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Keywords: Mitochondria, cancer, OPA1, virtual screening

The mitochondrial Dynamin-like protein Optic Atrophy 1 (OPA1) is a large GTPase involved in mitochondrial fusion, cristae remodeling, cytochrome c release and apoptosis. OPA1 upregulation has been increasingly identified as an exploitable vulnerability in cancer cells. From a previous screening of >10,000 drug-like molecules for inhibition of OPA1 GTPase activity, we identified MYLS22 as a promising hit. MYLS22 is not mitochondriotoxic but causes mitochondrial fragmentation and cristae remodeling, enhancing cytochrome c release following proapoptotic stimuli, recapitulating the effect of OPA1 downregulation in cells. Following a SAR analysis of MYLS22, multiple possible derivatives have been identified with increased water solubility. Three of these derivatives showed enhanced inhibitory effects on OPA1 GTPase activity *in vitro* and caused mitochondrial fragmentation in cells. To identify hits with further enhanced potency, solubility and overall availability of the leads, we generated a virtual, combinatorial library of 81,000 compounds. We docked these compounds to the GTPase domain of the currently available structure of OPA1 (PDB: 6JTG) and performed a virtual screening to discover second generation OPA1 inhibitors. We identified the most promising hits, synthetized them and analyzed their potency as OPA1 inhibitors in vitro and in cells. Our work identifies specific and potent OPA1 inhibitors with the potential to treat cancers where OPA1 is upregulated.

Proteomic insights into LRBA deficiency: investigating oxadiazole-based readthrough therapy

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Keywords: proteomic analysis, readthrough, LRBA deficiency, oxadiazoles

Inborn errors of immunity (IEIs) refer to a diverse group of genetic conditions caused by harmful mutations in single genes, leading to immune system dysfunction. These disorders can result in increased vulnerability to infections, autoimmune reactions, inflammation, allergies, bone marrow abnormalities, and certain cancers. IEIs are categorized into distinct groups based on their immune dysfunction. Among these disorders, some involve a loss of proper immune regulation, leading to uncontrolled immune activation and autoimmune symptoms. [1] A notable example of such a condition is LRBA (lipopolysaccharide-responsive and beige-like anchor protein) deficiency, a monogenic condition that disrupts key cellular functions like vesicular trafficking and autophagy. This autosomal recessive condition impairs the recycling of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), a critical regulator of immune responses. As a result, affected individuals experience immune hyperactivity, which can manifest as autoimmune cytopenias, chronic diarrhea, and lymphoproliferative complications. [2] Based on the clinical improvement observed in an LRBA-deficient patient undergoing ataluren treatment, [3] we aimed to investigate the effects of oxadiazole derivatives [4] as readthrough promoters targeting a specific nonsense mutation (c.5047C>T) in the LRBA gene. We conducted bottom-up proteomic analysis using label-free quantification (LFQ) on fibroblasts from a homozygous patient, with a heterozygous sibling cell line serving as a positive control. Cells were treated for 72 hours at 12 µM with various compounds, and comparative analysis revealed that heterozygous cells exhibited a broader and more dynamic shift in protein expression than homozygous cells. These findings highlight the complexity of LRBA deficiency and suggest that the response to readthrough therapy may be influenced by a variety of factors.

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Development of Nanobody Drug Conjugates (NbDCs) using SARS-CoV-2 as a model for targeted antiviral therapy

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Keywords: SARS-CoV-2, ADCs, NbDCs, payload, bioconjugation

The COVID-19 pandemic exposed the global inability to effectively manage emerging infectious diseases, particularly those caused by coronaviruses like SARS-CoV-2. These pathogens pose significant challenges for treatment with existing therapies, highlighting the need for novel strategies. This work introduces a new approach for treating future pandemics by developing anti-SARS-CoV-2 Nanobody Drug Conjugates (NbDCs) as efficient and tunable tools for viral infections. NbDCs represent a natural progression of Antibody Drug Conjugates (ADCs). To date, 15 ADCs have been approved for cancer treatment, [1] but there are no examples of antiviral ADCs. ADCs combine the specificity of monoclonal antibodies (mAbs) with the therapeutic effects of drugs, improving both the effectiveness and selectivity of the therapy. ADCs consist of three main components: a mAb that selectively binds the antigen; a drug, also called payload; and a linker, which can be either cleavable -releasing the payload under acidic or reducing conditions, or due to proteolytic enzyme activity- or non-cleavable, resulting in lysosomal degradation of the entire antibody-linker complex. Nanobodies (Nbs) are small antibody fragments from camelids that contain only a single variable domain (VHH), which is enough to bind antigens. [2] Using the anti-Spike protein nanobody, developed by Pierre Lafaye at the Institut Pasteur in Paris, the antiviral NbDCs act as a Trojan horse, selectively delivering the payload into infected cells without neutralizing the virus. Three different payloads have been selected for this study: Doxorubicin, a cytotoxic drug commonly used to evaluate the general activity of ADCs; [3] Niclosamide, a molecule active against specific viruses but not applied in clinics due to severe side effects; Remdesivir, an antiviral ProTide that has already been approved for use as an antiviral drug. [4] Regarding Doxorubicin and Niclosamide, these two payloads were connected to the Nb using a cleavable linker, which is stable in plasma, getting the payload release into the cell at pH 5.5. [5,6] Regarding Remdesivir, the payload was functionalized with a linker to ensure ProTide technology, allowing the selective activation of the nucleoside analogue exclusively in the infected cells, following ProTide metabolism. [4] The bioconjugation was performed by using the lysine residues of the Nanobody, which were coupled with the activated acid of the linker-payload system, resulting in the formation of the NbDCs with different Drug to Antibody Ratio (DAR) calculated by MALDI-TOF mass-spectroscopy. The NbDCs synthetized are showed in Figure 1.

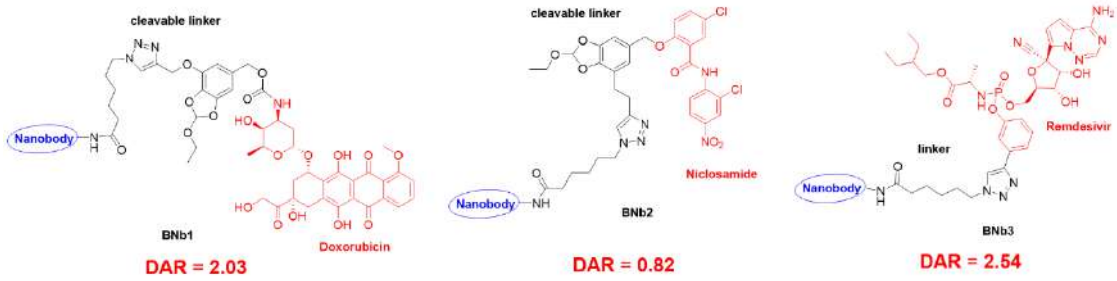

Figure 1: Nanobody Drug Conjugates (NbDCs) synthetized.

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Highly functionalized hybrid carbon nano-forms materials for photodegradation of pollutants from water

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Keywords: Photodegradation, carbon nano-forms, Zinc oxide

Water pollution caused by industrial dyes, pharmaceuticals, and organic contaminants represents a serious environmental challenge. Traditional treatment methods, including biological and chemical processes, often fail to effectively remove all classes of pollutants. Photocatalysis has emerged as a promising technology that leverages materials capable of absorbing solar light to generate reactive species for the degradation of organic contaminants. Among these, carbon-based nanostructures have garnered significant attention due to their unique structural, electronic, and optical properties. [1] In this study, we evaluate the use of nanomaterials in combination with the photocatalytic component like zinc oxide (ZnO). [2] Surface functionalization was optimized to enhance light absorption, charge separation, and pollutant interaction. We started using multiwalled carbon nanotubes (MWCNTs), highly functionalized with a dense network of polyamines (Figure 1), synthesized through various approaches to investigate their morphological characteristics. Following the reduction of imidazolium moieties, leading to the formation of secondary and tertiary amine groups, we developed a straightforward method to immobilize ZnO onto the nanomaterials at different concentrations. Characterization techniques such as TEM and XPS confirmed the successful integration and surface functionalization. Photodegradation tests, employing commercial organic dyes such as Toluidine Blue and Methyl Orange, are currently underway to evaluate the photocatalytic performance and recyclability of the materials.

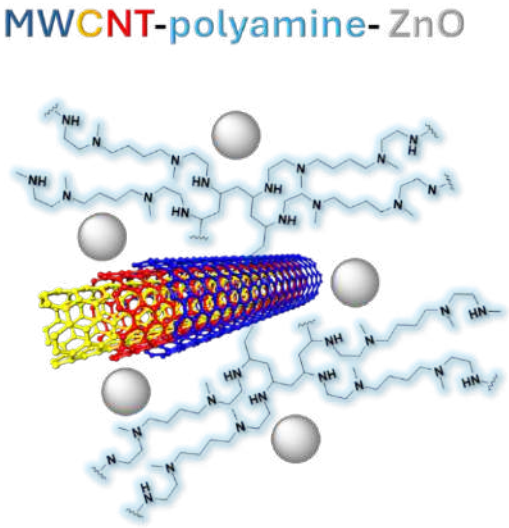

Figure 1: Graphical representation of a MWCNTs polyamine-functionalized and ZnO.

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Lung disorders treatment: Investigating the impact of a nasal nanoformulation in a murine model of pulmonary fibrosis

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Keywords: Nanomedicine, Pulmonary Fibrosis, Inflammation, Nanoparticles, Histopathology

Pulmonary fibrosis is a chronic disorder involving the lung parenchyma. It is characterized by the deposition of extracellular matrix, leading to lung remodelling. Although this alteration can be due to different factors, it always impairs respiratory function and, in idiopathic forms, is fatal. [1] Macrophages play a key role in fibrosis, as once activated, they secrete inflammatory or fibrotic factors. These cells are found near collagen-producing myofibroblasts, highly responsive to cytokines, indicating that inflammation and immune mechanisms contribute to fibrogenesis. [2] Therapeutic approaches to reduce fibrosis are still lacking due to the difficulty of reaching the target. Both intranasal administration and the use of nanocarriers might offer advantages in tackling this severe disease. [3,4] To this aim, the targeting of intranasally-injected Dexamethasone-loaded Avidin-Nucleic Acid-Nanoassemblies (ANANAS-Dex) has been evaluated in healthy mice and in a murine model of pulmonary fibrosis induced with bleomycin. The biodistribution of fluorescently labelled ANANAS-Dex was investigated through optical imaging technique and immunofluorescence analyses. To detect the hallmarks of fibrosis, a histopathological evaluation was performed. To correlate the carrier biodistribution and the release of the drug by ANANAS-Dex, the levels of Dexamethasone in plasma and lungs after a single administration were measured by HPLC–MS/MS. ANANAS-Dex were able to pass the lung surfactant barrier and rapidly penetrate inside the parenchyma, segregating in pulmonary macrophages in both healthy and fibrotic animals without diffusing into other organs. Pharmacokinetics revealed that the drug is released in the lungs, avoiding blood circulation and off-target accumulation. As expected, the instillation of free dexamethasone induced a marked spread in other organs. In addition, a relevant process of infiltration of inflammatory cells, disruption of alveoli, thickening of the alveoli walls, together with increased collagen deposition, were found in bleomycin-treated mice. These features are consistent with diffuse interstitial lung disease with an initial inflammatory stage, characterized by the activation of macrophages and neutrophils, followed by the development of fibrosis with subsequent abnormal deposition of collagen. Ongoing studies will give us a preliminary indication of a possible therapeutic effect of the treatment. Lung macrophages, which are involved in inflammatory disease onset and progression, have a strong ability to recognize, ingest, and degrade foreign materials, and can thus capture circulating nanoparticles, making these systems the ideal drug carriers for the controlled release in inflammatory-related diseases. In this context, they may represent a possible target for the development of new therapeutic strategies. Moreover, the targeted organ approach and steroid inclusion in ANANAS-Dex enhance the biosafety for translational applications.

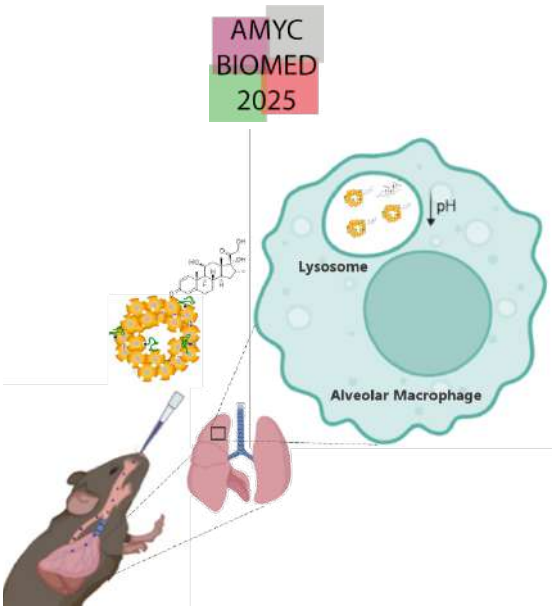

Figure: ANANAS nanoparticles as a novel drug delivery system for the treatment of lung disorders. Alveolar macrophages can uptake the dexamethasone-loaded nanoparticles and release the drug, avoiding off-target accumulation.

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Nanocrystals technology to enhance limitations of isoliquiritigenin

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Keywords: Nanocrystals, Isoliquiritigenin, Design of experiment, Nanomedicine.

Nanocrystals technology is a nanomedicine strategy that allows us to overcome the limits of many natural molecules such as their low water solubility and low bioavailability. By reducing the particle size down to the nanosize range, nanocrystals technology allows us to modify physico-chemical properties of natural molecules with beneficial effects on drug bioavailability. It is possible to classify molecules with poor solubility using the Biopharmaceutical Classification System (BCS). Many drugs, belonging to class II, are commonly formulated as nanocrystals because this strategy, by amorphization or reduction in particle size through nanonization techniques, could overcome solubility issues. [1] Nanocrystals (NCs) are defined as carrier-free drug particles. NCs in fact, are composed principally by drug surrounded by stabilizers, surfactants, or a mixture of both. Due to high drug concentrations, pharmacological activity of NCs is better than that of free-drug, and it allows the use of NCs in the clinic to be increased by reducing the dose. [2] In addition to the therapeutic purpose, nanocrystals technology can be applied for diagnostic scope. [1] In this work we selected isoliquiritigenin (ILQ) as a natural molecule with promising pharmacological proprieties. ILQ is a flavonoid derived from liquorice root that has gained significant interest within the scientific community due to various pharmacological properties, including antioxidant, anti-inflammatory, and anticancer effects. [3] However, the clinical application of ILQ is significantly hampered by its poor water solubility, leading to low bioavailability. [1] The goal of this study was to obtain optimized ILQ nanocrystals by using a solvent–antisolvent sonoprecipitation method through Design of Experiment (DoE). [4,5] In Optimal Custom Design, the concentration of surfactant (% w/v surfactant/aqueous phase) and the type of surfactant (Kolliphor P188, poly(vinylalcohol), and poly(vinylpyrrolidone)) were selected respectively as numeric and categorical factors. According to the selected independent and dependent (mean size (nm), polydispersity index (PDI), Zeta potential (mV)) variables, ten runs were obtained, and the formulations were characterized by Photon Correlation Spectroscopy. The obtained ILQ NCs showed a mean size in the range of 80 nm, and a PDI lower than 0.3, which is the limit accepted for qualitative formulations. The Zeta potential of ILQ NCs showed a maximum value of -40.3 ±0,569 mV. ILQ NCs optimization was performed using the 'desirability tool' provided by the DoE software, setting the factors in order to minimize mean size and Zeta potential. The optimized formulation will be further studied to investigate the role of ILQ NCs in metabolic syndrome by *in vitro* and *in vivo* studies.

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N,S-doped carbon nanodots conjugated with indoximod for a dual-mode approach in breast cancer immunotherapy

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Keywords: Breast cancer, nanomedicine, theranostics, carbon dots, immunotherapy

Despite the wide range of currently available therapies, breast cancer (BC) remains one of the most difficult malignancies to treat due to tumor heterogeneity, along with the occurrence of multidrug resistance and immune tolerance phenomena. [1,2] Addressing immunosuppression emerged as a promising therapeutic approach in BC treatment, leading to over 400 clinical trials. [3] However, the development of robust immunotherapies faces the complexity of immunoresistance mechanisms in the TME, leading to high variability in patients' responses. Combination therapy harnessing the synergistic effect of immunotherapy with conventional treatments led to better clinical outcomes, supporting the effectiveness of multi-target approaches. [4,5] Multifunctional nanoplatforms suit this purpose, offering new opportunities for targeted combination therapy. [6] Carbon nanodots (CDs), in particular, are promising nanosystems for precision cancer therapy as they can be engineered to hold inherent nanotoxicity toward cancer cells, besides enabling efficient targeted drug delivery within the TME. [7] Indeed, CDs present an ultrasmall diameter allowing their spread throughout the complex and dense TME, which often acts as a barrier to the penetration of nanomedicines. [8] Moreover, CDs present unique optical properties, including tunable multicolor fluorescent emission conferring self-trackability to these nanoparticles, which is valuable for bioimaging purposes, and many additional benefits including high water solubility, ease and low-cost synthesis, and versatile surface chemistry. [9] Despite the growing interest in CDs due to their particular characteristics, limited research has been reported to date on the use of these nanostructures to elicit innate and adaptive antitumor immune responses, leaving this strategy still underexplored. We proposed to develop multifunctional CDs that exhibit intrinsic nanotoxicity against cancer cells and drive effective antitumor immune responses in breast cancer. To this aim, we leveraged the inherent anticancer activity of our N,S-doped CDs, which demonstrated to selectively tackle breast cancer through a mechanism involving increased expression of reactive oxygen species (ROS). [10] Since the overproduction of ROS leading to cell death has been associated with enhanced antitumor immune response in literature, [11] we proposed to conjugate N,S-doped CDs with the immune adjuvant indoximod (IND), which has achieved encouraging results in combination therapy regimens. [12,13] The anticancer properties of CDs-IND were investigated using both *in vitro* and *in vivo* breast cancer models followed by extensive TME characterization. CDs-IND effectively enhanced the immune response against breast cancer *in vivo*. Finally, we demonstrated the similar effectiveness of CDs-IND administrated locally and systemically to ensure the optimal delivery and efficacy of the developed nanotechnology (Figure 1).

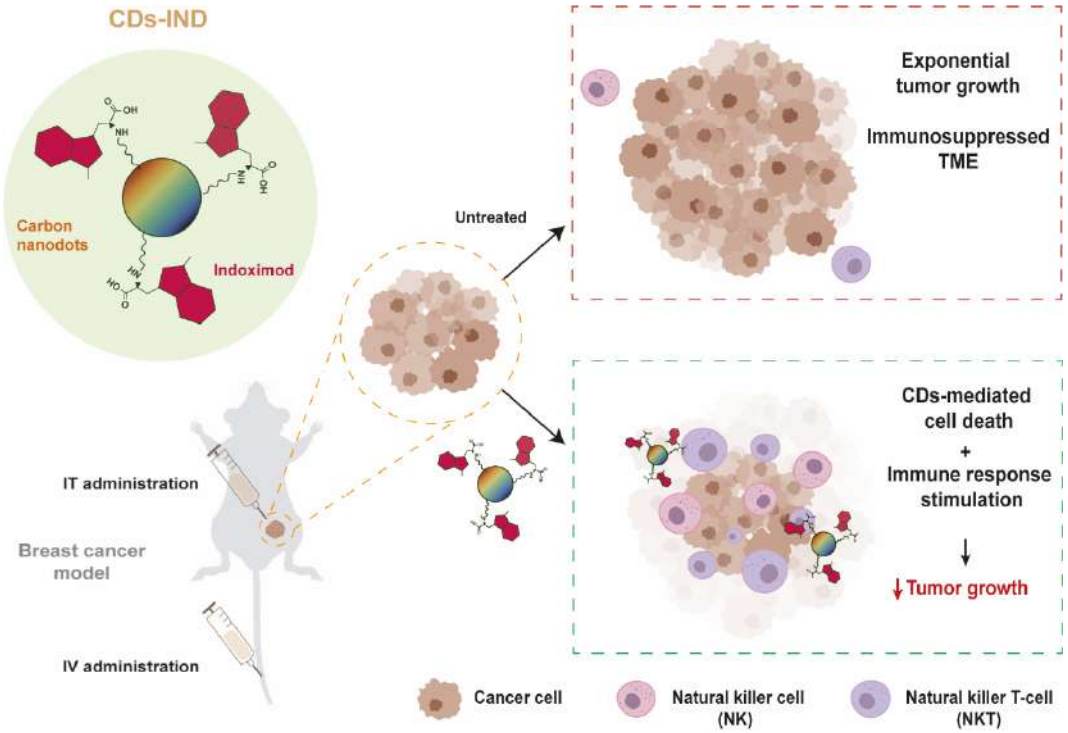

Figure 1: Graphical illustration of CDs-IND structure and antitumoral activity.

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Media milling optimization for rapid extraction of nanovesicles from lemon

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Keywords: Lemon-derived nanovesicles, Media milling, extracellular vesicles.

Plant-based extracellular nanovesicles (ENVs) have emerged as promising candidates for various biomedical applications due to their biocompatibility and intrinsic bioactivity. [1] Current methods for isolating EVNs result in low yields and involve lengthy procedures. This study aimed to optimize ENVs extraction using a media milling technique. Lemon pulp was chosen as the raw material because of its abundance and high potential for nanovesicle production. We systematically varied key parameters of the media milling process, including bead size and milling time, and used a dual asymmetric speed mixer to optimize the size and polydispersity of the nanovesicles. Dynamic light scattering (DLS) was used to monitor the size distribution and polydispersity of the resulting nanovesicles. Our results indicate that optimized media milling conditions can significantly increase the efficiency of LENV isolation, yielding nanovesicles with a narrow size distribution and reducing the polydispersity. Further characterization of the morphology of the isolated LENVs was conducted using transmission electron microscopy (TEM) imaging. In conclusion, this optimized media milling approach (Figure 1) offers a rapid and scalable method for producing LENVs, potentially facilitating their application in various fields. Moreover, this methodology could be adapted to other plants, expanding the potential applications of this technology.

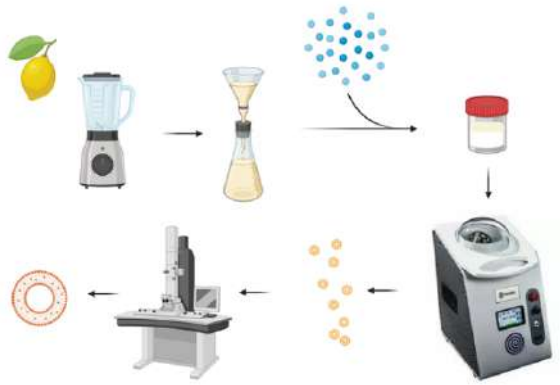

Figure 1: Schematic procedure of ENVs extraction and optimization

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Self-nanoemulsifying drug delivery systems (SNEDDS) improve curcumin's impact on retinal protection

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Keywords: ocular drug delivery systems, nanoemulsion, curcumin, Sirtuin-1, diabetic retinopathy

Sirtuin-1 (SIRT1), a histone deacetylase enzyme expressed in ocular tissues and localized intracellularly, plays a pivotal role in numerous physiological processes, including protection against oxidative stress and inflammation. The observed correlation between decreased SIRT1 levels and diabetic retinopathy (DR) has prompted interest in exploring natural therapeutic agents that function as SIRT1 activators. [1] Polyphenolic compounds have been shown to upregulate SIRT1 expression; however, their therapeutic potential is constrained by poor solubility, low bioavailability, and instability under physiological conditions. Curcumin (CUR), a polyphenol known to enhance SIRT1 expression, has emerged as a promising candidate for DR treatment. Nevertheless, achieving effective delivery of CUR to deeper ocular tissues, particularly the retina, remains a major challenge due to its limited solubility and poor ocular penetration following topical administration. To address these limitations, the development of self-nanoemulsifying drug delivery systems (SNEDDS) for topical ocular delivery of CUR represents a novel strategy. A blank SNEDDS formulation was optimized using Design of Experiments (DoE) software, which facilitated the construction of a ternary phase diagram to identify the emulsification zone, and Response Surface Methodology (RSM) to optimize the formulation for topical ocular delivery. [2] The optimized SNEDDS, loaded with CUR, were subsequently prepared and characterized following reconstitution with simulated tear fluid (STF) at a 1:10 ratio. The formulation demonstrated small droplet size, high clarity, and the absence of drug precipitation upon resuspension in STF. Compared to free CUR, CUR-loaded SNEDDS exhibited improved stability and a more favourable release profile under simulated ocular conditions. In vitro analysis of mucoadhesive properties indicated that CUR-SNEDDS, modified with a cationic lipid, exhibited enhanced interactions with mucin, suggesting improved ocular retention. Cytotoxicity assays confirmed that CUR-SNEDDS maintained the viability of human corneal epithelial (HCE) cells and retinal pigment epithelial cells (ARPE-19) at concentrations up to 3 µM. Furthermore, in an oxidative stress model using ARPE-19 cells exposed to hydroquinone (HQ), CUR-SNEDDS displayed superior antioxidant activity compared to free CUR. Cellular uptake studies revealed significantly greater accumulation of CUR within retinal cells following exposure to CUR-SNEDDS, even at lower concentrations, compared to unformulated CUR. This is a critical achievement for facilitating the intracellular induction of SIRT1. Notably, CUR-SNEDDS at concentrations of 0.1 µM and 0.5 µM effectively upregulated SIRT1 expression. [3] Overall, the cytocompatibility, increased antioxidant effects, and enhanced cellular uptake of CUR-SNEDDS highlight their potential as effective delivery systems for improving CUR retinal protection in DR.

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Single-Step Monitoring of Bioavailable Therapeutic Antibodies Using a Protein-DNA Nanoswitch Platform

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Keywords: Anti-Drug Antibodies, Therapeutic Drug monitoring (TDM), Monoclonal Antibodies (mAbs), DNA nanotechnology, DNA switch

Monoclonal antibodies (mAbs) are a fundamental component of modern therapeutics, yet current monitoring approaches often fail to reflect their true bioavailability. To address this critical gap, we developed a cutting-edge protein-DNA nanoswitch platform designed for precise, real-time assessment of bioavailable mAbs in complex biological matrices. [1] This modular sensor integrates whole TNF-alpha protein within a ssDNA oligonucleotide, creating a multi-epitope recognition system capable of detecting clinically important mAbs, including Infliximab and Adalimumab, directly in human serum. The platform exhibits robust sensitivity within the therapeutic range (2.4–19 µg/mL), enabling straightforward, one-step quantification without complex sample preparation. Crucially, the nanoswitch enables the selective detection of bioavailable drug, as demonstrated by its ability to measure decreased levels of Infliximab in the presence of anti-drug antibodies (ADAs). This capacity to selectively measure the bioavailable fraction has significant implications for personalized medicine, supporting tailored dosing strategies and improving treatment outcomes for patients on long-term biologic therapies. The versatility and adaptability of this multi-epitope nanosensor make it a promising candidate for broader pharmacokinetic studies and real-world therapeutic drug monitoring applications.

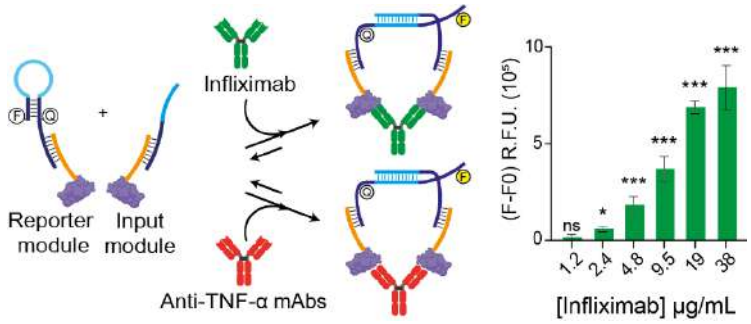

Figure: Schematic representation of the Protein-DNA nanoswitch, a modular biosensing platform comprising a Reporter Module and an Input Module. The system leverages a proximity-induced conformational change upon target antibody recognition, facilitating beacon activation and fluorescence emission. The fluorescence intensity is directly proportional to the target concentration, enabling quantitative analysis. This platform has been validated for the direct detection and quantification of monoclonal antibodies in human serum.

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NIR-Responsive Antimicrobial Hydrogels Based on Gellan Gum and bis-MPA Polyester Dendrimers

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Keywords: wound healing, gellan gum, antimicrobial dendrimer, xerogel, antibacterial, radical scavenger

The skin acts as a protective barrier, but inadequate wound management can lead to infections and serious complications. [1] The increasing antimicrobial resistance (AMR) [2] requires new therapeutic strategies and multifunctional wound dressings that can manage exudate and scavenge radical scavenging oxygen species (ROS) scavenging. [3] Innovative materials such as hydrogels and sponges are emerging as promising alternatives. [4] In particular, gellan gum (GG) is an interesting candidate for the development of these systems. [5] Antimicrobial bis-MPA dendrimers offer versatility and low toxicity. [6] Furthermore, polydopamine (pDA) supports healing due to its photothermal and antioxidant properties. [7,8] In this context, multifunctional hydrogels composed of GG-EDA (ethylenediamine-functionalized gellan gum) with in situ synthesized pDA and enriched with bis-MPA dendrimers (TMP-G1-$[Cys]_6$ and TMP-G2-$[Cys]_{12}$) have been developed. The GG-EDA@pDA hydrogels at 25°C and 37°C showed a viscoelastic behavior typical of physical hydrogels; they also demonstrated the photothermal conversion capability under NIR irradiation (810 nm), reaching temperatures of 42-45°C in 6-8 min with 1.27 W/cm^2. The $ABTS^{\bullet+}$ colorimetric assay showed that both GG-EDA@pDA and starting GG-EDA possess a dose-dependent antioxidant capacity; the presence of pDA increased the activity reaching 100% radical scavenging already at a concentration of 1.5 mg/ml. To impart antibacterial activity, TMP-G1-$[Cys]_6$ and TMP-G2-$[Cys]_{12}$ dendrimers were adsorbed onto the freeze-dried hydrogel-derived sponges via noncovalent interactions, including electrostatic interactions with the carboxyl groups of GG-EDA and cation-π interactions with the aromatic rings of pDA. The dendrimers, featuring 6 and 12 protonated amino groups at physiological pH, respectively, disrupt bacterial membranes, enhancing antibacterial activity. Due to the noncovalent interactions with both GG-EDA and pDA, the dendrimers are retained differently within the sample. This difference in retention influences their antimicrobial activity against Pseudomonas aeruginosa and Staphylococcus aureus. In particular, the NIR-induced photothermal effect was shown to play a crucial role in triggering the activity of the sample loaded with the most retained dendrimer, which possesses the highest number of terminal positive charges. The high absorption capacity of physiological fluids makes these materials ideal for wound exudate management. Furthermore, their resistance to hydrolytic degradation can be exploited to reduce the frequency of dressing changes, potentially improving patient comfort. Dendrimer-loaded samples demonstrated low cytotoxicity towards human fetal dermal mesenchymal stromal cells (FD-MSCs) and human epidermal keratinocytes (HaCaT), suggesting that GG-EDA@pDA+TMP-G1-$[Cys]_6$ and TMP-G2-$[Cys]_{12}$ hydrogels may be promising candidates for the treatment of infected wounds.

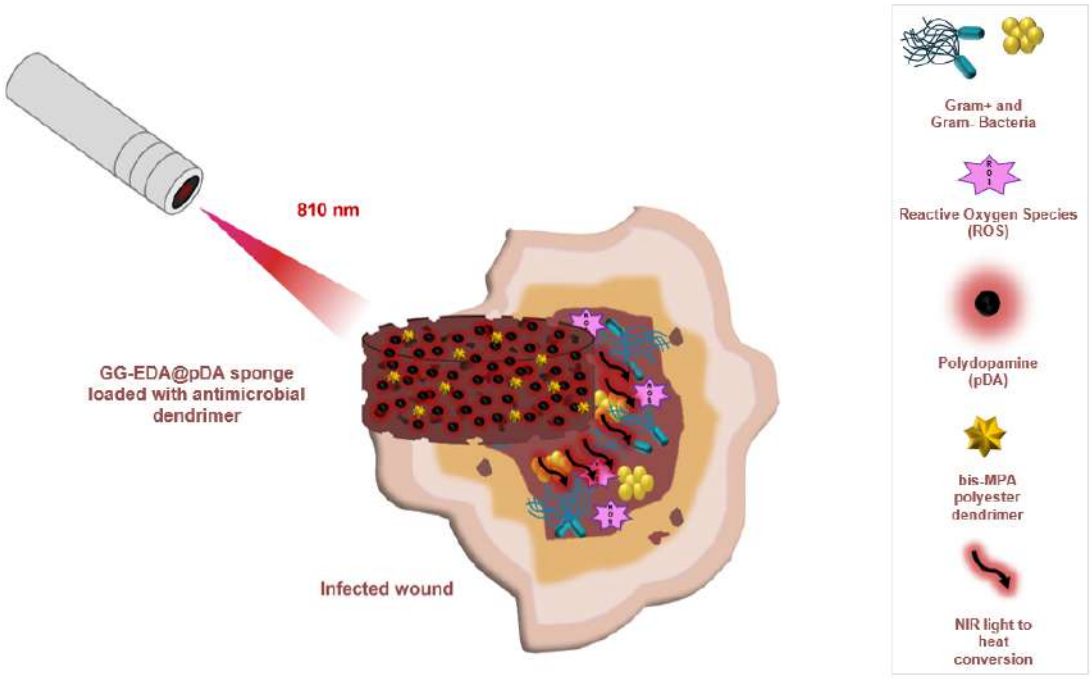

Figure: NIR-Responsive antimicrobial GG-EDA@pDA sponge with bis-MPA Polyester.

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Rapid determination of solar UV filters in human whole blood, plasma and urine using an FPSE-HPLC-PDA method

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Keywords: UV filters, FPSE-HPLC-PDA, green analytical chemistry, method validation

Prolonged human exposure to solar UV radiation may have adverse effects on healt. In order to prevent solar UV radiation exposure, solar UV filters are incorporated in sunscreens formulations and in daily-use cosmetics, such as face creams, makeup formulations, shampoos, after shave lotions and other products. These solar UV filters are considered emerging pollutants as they are present in several matrices. Due to their presence in cosmetic products, they can be absorbed through the skin after topical application; benzophenone derivatives bind to plasma proteins and can bio-accumulated in the organism, because of their lipophilic nature. In order to investigate UV filter presence, the most commonly used techniques is a detector coupled to LC based on ultraviolet/visible spectroscopy (UV/Vis). In this work the HPLC-PDA instrument configuration coupled to fabric phase sorptive extraction (FPSE) device was used to simultaneously separate and analyze six benzophenone derivative UV filters in human whole blood, plasma and urine. The method validation was carried out according to the International Guidelines in order to evaluate the limit of detection (LOD), limit of quantification (LOQ), linearity, intra- and inter-day trueness and precision, selectivity, recovery, and long term stability of six benzophenone type UV filters in human whole blood, plasma and urine samples. When comparing this chromatographic separation method with others in the literature, it can be affirmed that the present method is very simple and cheap, elution occurs in isocratic mode and the chromatographic run lasts only 25 minutes, a time that allows the complete separation of the largest number of benzophenone derivative UV filters without any compromise in peak resolution. The new approach, based on FPSE-HPLC-PDA, has been used for the simultaneous detection of six UV filters in human whole blood, plasma and urine samples. The new FPSE-HPLC-PDA validated method is simple, fast, environmentally friendly, allows direct analysis on whole blood without any previous pre-treatment and allows a significant decrease in organic solvents consumption in accordance with the principles of Green Analytical Chemistry (GAC). All this is advantageous in terms of the number of compounds analyzed, inexpensiveness and easiness of the instrumentation used, chromatographic run times and the speed of the sample pre-treatment phase. This work highlights that by applying innovative extraction and clean up procedures before instrumental analysis using configurations such as HPLC-PDA, it is possible to validate methods that demonstrate analytical performance comparable to more expensive and complex instrumentations (e.g. LC-MS/MS). [1]

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Few-Molecule Detection via SPR Enabled by pH Conditioning

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Keywords: plasmonic sensors, single-molecule sensing, single-molecule with a large transistor (SiMoT), surface probing techniques, surface-plasmon resonance

DNA can be easily amplified through replication, making it possible to detect even a single target copy. In contrast, achieving similar sensitivity for proteins in immunoassays remains challenging. Surface plasmon resonance (SPR) typically detects proteins at concentrations around 10^{-9} molar. [1] This study demonstrates plasmonic single-molecule assays for both proteins and DNA, reaching detection limits as low as 10^{-20} molar (1 ± 1 molecule in 0.1 mL), even in human serum, within just one hour. This represents an improvement of eleven orders of magnitude compared to conventional SPR limits. The single-molecule SPR assay is enabled by a millimeter-wide surface functionalized with a physisorbed biolayer containing trillions of recognition elements (such as antibodies or protein–probe complexes) that undergo pH conditioning (acidic or alkaline). Potentiometric and surface imaging experiments reveal a previously unexplored amplification process within the biomaterial. [2] This amplification occurs through pH conditioning, which drives the biolayer into a metastable state, leading to self-propagating aggregation of partially misfolded proteins after single-affinity binding. This, in turn, causes an electrostatic rearrangement and a charge displacement of approximately 1.5e per 102 recognition elements. These findings suggest exciting new possibilities for SPR-based biosensing at the physical detection limits, offering potential for advanced point-of-care plasmonic systems. [3]

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Integrating microfluidics and plasmonics for capturing and detecting alterations in genomic DNA from circulating tumor cells

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Keywords: Liquid biopsy, Surface Plasmon Resonance, Microfluidics, Superparamagnetic particles, Cancer diagnosis

Cancer remains one of the leading causes of death worldwide, necessitating innovative approaches for early diagnosis and timely treatment. In this context, liquid biopsy has emerged as a minimally invasive procedure for diagnosis and monitoring, enabling the detection and analysis of tumor-derived biomarkers directly from bodily fluids. However, the low abundance of tumor-specific biomarkers, particularly in early-stage cancer patients, necessitates the development of highly sensitive and reliable detection methods. [1] This work focuses on the development of an integrated microfluidic and plasmonic platform for liquid biopsy, [2] leveraging the peculiar capabilities of surface plasmon resonance imaging (SPRI) when combined with functionalized beads to enhance detection sensitivity. [3] A microfluidic device was designed and tested to perform the pre-analytical steps required to isolate DNA from tumor cells, minimizing sample volume and handling. The DNA is then transferred to the SPR imaging fluidic system for highly sensitive, amplification-free detection. Various devices were designed and tested to capture the target DNA using superparamagnetic beads functionalized with peptide nucleic acid (PNA) probes. A magnetic field is incorporated into the microfluidic device to immobilize the beads, facilitating efficient washing and the removal of impurities from liquid biopsy samples. Preliminary fluorescence-based evaluations confirmed the interaction between labelled target DNAs and the functionalized beads. Pilot SPRI experiments were conducted based on previous research. [4] These experiments aimed to detect G13D KRAS-mutated genomic DNA extracted from LoVo cells, which were derived from the tissues of patients with colorectal adenocarcinoma. PNA probes were used for SPRI detection due to their unique properties, allowing for the design of specific and highly sensitive assays. [5] By minimizing sample volumes and providing high sensitivity, this method has significant potential for application in early diagnosis based on circulating tumor cells. Detecting these cells is particularly challenging due to their low abundance in early-stage patients.

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The effect of metal ion coordination on the photodynamic activity of chlorins for Photodynamic Therapy

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Keywords: Chlorins, Photodynamic activity, Density Functional theory, Spin Orbital Coupling

Photodynamic therapy (PDT) is a cutting-edge, minimally invasive treatment that has gained significant attention in oncology due to its success in combating various cancers. [1] Beyond cancer, PDT's versatility extends to addressing bacterial, [2] and viral infections. [3] PDT operates by administering a photosensitizer (PS), a light-sensitive agent that absorbs specific wavelengths of light to produce reactive oxygen species (ROS). These ROS selectively destroy cancer cells while sparing healthy tissues, offering a targeted and less toxic alternative to conventional treatments. [4] PDT has several advantages: (i) tumor-specific targeting due to preferential PS accumulation, (ii) localized activation via light exposure, (iii) minimal side effects, (iv) destruction of tumor-associated vasculature critical for growth, and (v) immune system stimulation that may lead to long-term anti-tumor immunity. However, first-generation PSs face limitations such as low light absorption and aggregation issues. Second-generation PSs like chlorins address these drawbacks with improved light absorption in the 650–850 nm range, higher ROS generation efficiency, and reduced aggregation. [5] The therapeutic potential of chlorins can be further enhanced through metallation, where metal ions are integrated into their structure. This modification improves stability, photophysical properties, and intracellular distribution. [6] In this study, computational methods such as Time-Dependent Density Functional Theory (TD-DFT), Spin-Orbit Coupling (SOC), and intersystem crossing rate calculations were employed to evaluate how different metal complexes influence the photodynamic activity of chlorins. Results indicated that metal ions like platinum(II/ IV) and osmium(II) enhance SOC values, aligning with existing literature. [7] This research underscores the potential of metallochlorins as advanced photosensitizers for PDT, paving the way for more effective cancer therapies.

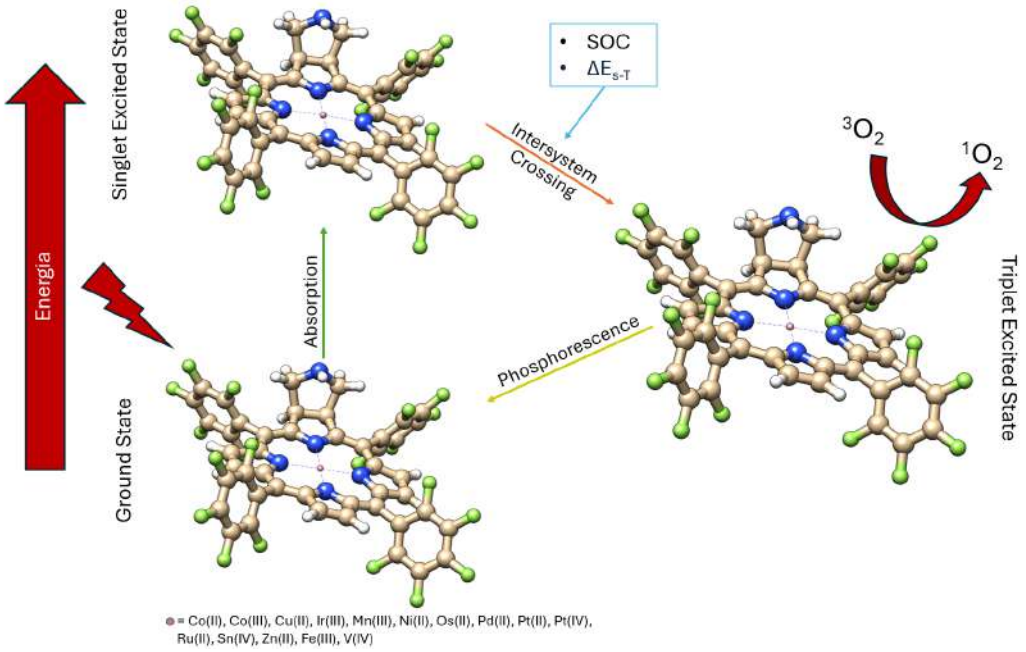

Figure 1: Representation of the photodynamic process in the production of singlet oxygen.

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Mitochondrial $K_V1.3$ inhibitors: A New Frontier in Anticancer Therapy

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Keywords: cancer, apoptosis, mitochondrial $K_V1.3$, triphenylphosphonium

Mitochondrial $K_V1.3$ channel is located in the mitochondrial inner membrane (IMM) and belongs to the voltage-gated potassium channel $K_V1.x$ subfamily. Mito$K_V1.3$ are involved in mitochondrial potassium cycle and in the regulation of the intrinsic apoptotic pathway.[1] It has emerged as a promising target for anticancer therapy due to a correlation between overexpression of mito$K_V1.3$ and cancer development. [2,3] In our research, we identified, as a starting point, a parent compound with potent cytotoxic activity. It presents a benzamide scaffold, [4,5] a four carbon linker chain and triphenylphosphonium (TPP^+) as mitochondria-targeting moiety (MTM). We explored structural modifications to enhance its activity, we synthesized and evaluated different analogues with variations in the linker chain length (three to six carbons) and modifications in the mitochondria-targeting moiety (MTM), specifically TPP^+ and its substituted derivatives. The new inhibitors were screened for antiproliferative activity in pancreatic cancer COLO-357 and Ewing sarcoma SK-N-MC cell lines, demonstrating significant cytotoxicity and cell viability reduction. Among all tested compounds, *cis*-24 emerged as the most promising one. Patch-clamp experiments confirmed its potent inhibition on mito$K_V1.3$. Moreover, it caused mitochondrial membrane depolarization and induced apoptosis via caspase 3/7 activation in COLO-357 cell line. Further investigations revealed that *cis*-24 selectively inhibited tumour cell growth without affecting growth of non-tumour hTERT-RPE1 cells, activated the permeability transition pore (PTP) rather than significantly increasing reactive oxygen species (ROS), distinguishing it from other mito$K_V1.3$ inhibitors. To assess the compound's efficacy beyond 2D cultures, *cis*-24 was evaluated in 3D spheroids models of PANC-1 and COLO-357. It induced strong anti-proliferative and cytotoxic effects, particularly in COLO-357 spheroids, where significant impact was observed even at low micromolar concentrations. Finally, in an *in vivo* zebrafish xenograft model of Ewing sarcoma SK-N-MC, *cis*-24 significantly inhibited tumour growth at 2 μM, demonstrating promising efficacy in an *in vivo* model. These findings highlight the potential of *cis*-24 as a novel mitochondrial $K_V1.3$ inhibitor for cancer therapy, with significant anticancer activity. The observed efficacy in both *in vitro* and *in vivo* models suggested that *cis*-24 could serve as valuable lead compound for further development in this field.

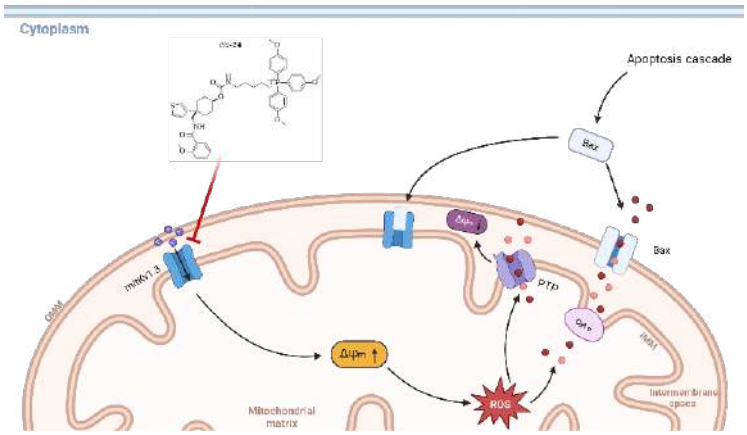

Figure: *cis*-24 inhibits mitoKv1.3 in cancer cells, increasing permeability transition pore (PTP) activation, triggering apoptosis and ultimately leading to cell death.

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Multivariate Quality Control and Machine Learning for early pancreatic cancer diagnosis using the SiMoT platform

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Keywords: SiMoT, pancreatic cancer, machine learning, quality control

The Single-Molecule-with-a-large-Transistor (SiMoT) [1] platform is an innovative lab-based technology that enables single-molecule detection of genomic and proteomic biomarkers. [2,3] Pancreatic cancer classification is associated with three specific molecular targets: the MUC1 and CD55 proteins, and the KRAS gene, particulary the KRASG12D mutation. [4] In a previous study, [5] a total of 47 patient-derived fluid samples were analyzed. For each sample, the simultaneous detection of MUC1, CD55, and KRASG12D was performed, along with seven negative control experiments. The patient cohort was subsequently expanded, and a multivariate quality control strategy based on Principal Component Analysis (PCA) [6,7] was introduced to improve the reliability of the SiMoT platform. This approach enables the identification of experiments exhibiting abnormal biosensor behavior, which are excluded from further analysis. The quality control process utilizes current differences between sensing and baseline conditions across all reference gates and negative controls, leveraging the lateral gate architecture of the SiMoT device to monitor sensor stability. From the transfer curves acquired using the SiMoT platform, three features were extracted per biomarker. These features served as input for the development of a machine learning-based classifier for pancreatic cancer diagnosis. PCA was first employed for exploratory multivariate data analysis and visualization of clustering patterns. A k-nearest neighbors (k-NN) classifier was then trained to distinguish between high-grade mucinous cysts, low-grade mucinous cysts, and non-mucinous lesions. The classifier was trained on a dataset comprising 50 patient samples and validated on an independent external test set of 29 samples analyzed in a double-blind manner. The combined analysis of the three SiMoT markers across all 79 patients resulted in a diagnostic sensitivity of 92.1% and a diagnostic specificity of 100% for the identification of mucinous neoplasms.

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New Horizons in TP53 Nonsense Mutations Treatment: Unveiling Novel Translational Readthrough-Inducing Drugs for Cancer Therapy.

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Keywords: P53, Nonsense mutation, Cancer, Translational readthrough, Computer Aided Drug Design.

Nonsense mutations occur when a point mutation converts an amino acid-coding triplet into a premature stop codon, leading to the formation of a Premature Termination Codon (PTC). This results in decreased cytosolic mRNA levels and premature termination of translation, producing a truncated and non-functional protein. Nonsense mutations account for approximately 11% of genetic diseases and affect 12% of tumor suppressor genes, including TP53, one of the most frequently mutated tumor suppressor genes in human cancers. [1] The TP53 gene encodes p53, a transcription factor known as "*the guardian of the genome*," which plays essential roles in DNA repair, apoptosis induction, senescence, and antiproliferative activities. [2] TP53 mutations lead to the loss of tumor suppressor function, contributing to cancer development. [3] Notably, over 50% of human cancer cases are characterized by mutations in TP53, with approximately 10% of these being nonsense mutations, underscoring the crucial need for novel therapeutic strategies. [4] Several promising approaches to p53-based treatment have emerged, offering hope that TP53 alterations may finally be considered treatable. Among these, translational readthrough (RT)-based therapy has gained significant attention. This approach pharmacologically suppresses premature stop codon at the post-transcriptional level by using Translational Readthrough-Inducing Drugs (TRIDs), thereby potentially restoring the expression of full-length p53 protein and reactivating its tumor-suppressing functions. [5] In this study, using an optimized drug design strategy with extensive application of Computer-Aided Drug Design (CADD), we identified a new chemical scaffold containing a 1,2,4-triazolic heterocyclic core capable of restoring the expression of full-length p53 protein. We developed a novel pharmacophore model using a ligand-based approach to screen new potential TRIDs. The synthesized compounds were tested for translational readthrough activity using an H1299 p53(-/-) R213X cell-based reporter system. The best-performing molecules, selected based on in-silico and in-vitro toxicity assessments, and in-silico ADME (Absorption, Distribution, Metabolism, Excretion) profiling, were further evaluated for their effectiveness in producing full-length p53 protein under stress conditions and restoring its functionality. We identified four 1,2,4-triazolic derivatives able to restore p53 expression and functionality, with no observable in-vitro toxicity and that possess in-silico ADMET profiles, making them promising candidates for further development in cancer therapy.

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Innovative chemophototherapy strategies for cancer

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Keywords: Anticancer, Chemophototherapy (CPT), Combination therapy, Virtual screening

Despite its high efficacy, two factors usually limit the clinical use of chemotherapy: severe side effects and the development of chemoresistance. Combination therapies can bypass these limits. Chemophototherapy (CPT) integrates photodynamic therapy (PDT) with chemotherapy. Due to the synergistic effect of the two approaches, CPT reduces the risk of recurrence, overcomes tumor drug resistance, and allows for lower chemotherapy dosages, decreasing the related adverse effects. CPT strategies included simultaneous administration of a chemo drug and a photosensitizer (PS), the development of new drugs in which the structure of a chemo drug and a PS are chemically linked together, and the use of drug delivery systems loaded with both a chemo drug and PS. [1] We developed two innovative approaches for CPT: i) Simultaneous transport of PS and chemotherapeutic drugs using proteins. Protein and protein nanoparticles provide biocompatible platforms for the simultaneous delivery of chemotherapeutic agents and photosensitizers. Human serum albumin (HSA) and different plasma carrier proteins, identified by a virtual screening approach, were used by us to disperse a chemotherapeutic drug or a PS in solution [2], improving their anticancer activity. Protein nanoparticles have also been successfully used to deliver chemotherapeutic agents, such as Abraxane® (an albumin nanoparticle-paclitaxel-based treatment, FDA approved). PS can be included in this formulation and the final CPT treatment strongly improves the final outcomes; ii) Exploitation of the intrinsic photosensitizing properties of some chemotherapeutic drugs, like doxorubicin, to enhance their anticancer activity upon light irradiation. [3] A computational screening identified Epirubicin, Daunorubicin, Mitoxantrone, and Pixantrone as the best candidates to act like photosensitizers. Photoactivation of the identified compounds produces peroxides and singlet oxygen in a light-dependent manner and leads to an increase in their cytotoxic activity (reduction of the IC50 value up to an order of magnitude) due to the generated oxidative stress. Following photoactivation, different cell death modalities were activated.

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Waterborne polyurethane-based electroresponsive platforms for controlled chemotherapy delivery

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Keywords: Electroresponsive drug release, localized cancer therapy, waterborne polyurethane, PEDOT, redox-responsive materials.

Systemic chemotherapy is often limited by severe off-target toxicity, leading to dose limitations and reduced therapeutic efficacy. Electrically controlled drug release systems [1–3] offer a promising strategy for localized, on-demand chemotherapy, minimizing side effects and enhancing therapeutic outcomes. To address these challenges, in this work a glutathione integrated waterborne polyurethane (WPU-GSSG) [4] has been engineered as a multifunctional biomaterial, capable of responding to both electrical and redox stimuli, ensuring precise and selective drug delivery. WPU was designed as a redox-responsive polymer by incorporating oxidized glutathione as a chain extender, introducing cleavable disulfide bonds that enable drug release in response to intracellular glutathione, overexpressed in tumor cells. Poly(3,4-ethylenedioxythiophene) (PEDOT) was incorporated to introduce electrical responsiveness to the drug delivery system. PEDOT nanoparticles (NPs) were synthesized and loaded with tamoxifen (TMX) and metformin (MET) to enable electrostimulated drug release. WPU/PEDOT-based platforms were developed in different forms, including electroconductive membranes, sponges and hybrid hydrogels, thus integrating natural polymers for enhanced biocompatibility. These materials were extensively characterized for their mechanical properties, electrochemical behavior, and drug release kinetics under redox and electrical stimulating conditions. PEDOT NPs demonstrated the ability to selectively release TMX and MET on-demand upon electrical stimulation. WPU-GSSG/PEDOT membranes exhibited high conductivity and tunable mechanical properties, enabling precise, electrically controlled release. The electropolymerization of PEDOT on the membrane surface significantly enhanced electroconductivity, improving the efficiency of electrically triggered drug desorption. WPU-GSSG/PEDOT sponges displayed a highly porous structure, efficient electrical actuation, and excellent elastic stiffness, reaching an elastic modulus of 680 kPa and a compressive stress of 233 kPa, making them highly suitable for localized chemotherapy applications. Hybrid hydrogels provided a biocompatible, electroresponsive matrix with improved mechanical resilience, facilitating controlled drug release. The dual-responsive behavior of these materials highlights their potential for synergistic anticancer therapy by integrating redox-triggered and electrostimulated release mechanisms. The integration of WPU with PEDOT enables a multifunctional, dual-responsive drug delivery system, bridging electroconductivity with tumor-specific redox responsivity. This platform allows for precise, on-demand drug release, significantly reducing systemic toxicity while enhancing therapeutic efficacy. These findings highlight the potential of WPU-based materials for personalized, localized chemotherapy, opening the way for next-generation responsive biomaterials in cancer treatment.

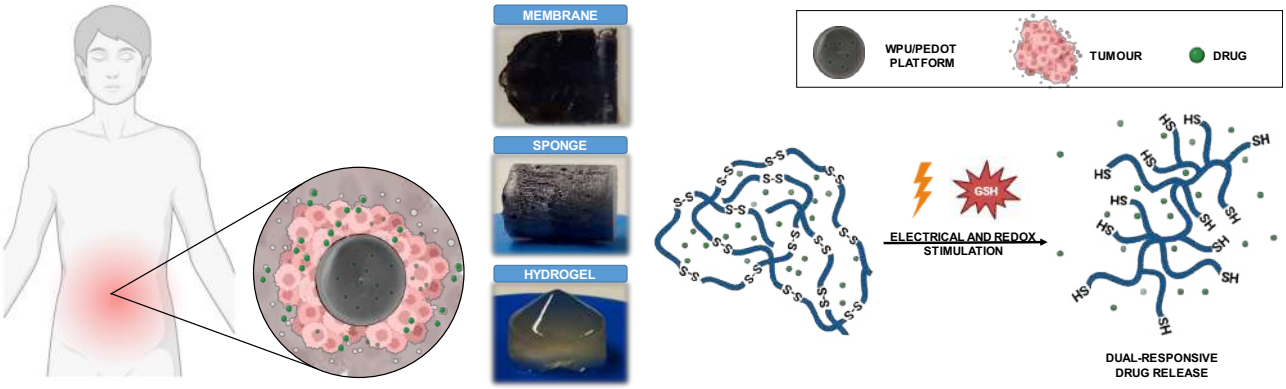

Figure: Schematic representation of dual-responsive WPU-GSSG/PEDOT-based systems for electro- and redox-triggered drug release.

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Fighting Cancer at the Proton Gate: New H_V1 Proton Channel Inhibitors

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Keywords: voltage-gated proton channels, virtual screening, patch-clamp, inhibitor, anticancer

Voltage-gated proton channels (H_V1) are proton-selective voltage-dependent channels that have been found in various mammalian and cancer cells, playing a key role in preventing intracellular acidification. They have been linked to tumours development, neuroinflammatory diseases, immune disorders and infertility. [1] In particular, H_V1 overexpression disrupts pH homeostasis and is closely associated with cancer progression. Indeed, in physiological conditions, at the resting membrane potential, the channels are closed; however, pathological conditions can trigger their opening even at the resting membrane potential, creating an acidic microenvironment in which tumour cells can adapt extremely well, while immune cells functions are impaired. [2] The aim of our work is to discover and evaluate new H_V1 inhibitors. Currently, there are no selective inhibitors specific for H_V1 channels. A selective inhibitor would allow us to modulate the acidic tumor microenvironment and to better understand the role of H_V1 in tumours and other relevant diseases. An open structure of the human H_V1 channel was used to perform virtual screening (VS) of commercial and in-house libraries of compounds. [3] Compounds were docked to the binding site of some known guanidine derivatives, such as 2-guanidinobenzimidazole (2GBI), on the voltage-sensing domain. [4] VS results were evaluated, and a series of most promising hits were selected to be tested by manual patch-clamp electrophysiology in Chinese Hamster Ovary (CHO) cells transiently transfected with human H_V1. With an IC_{50} value of 8.5 µM, compound 13 exhibited a significant block of the proton current. Six additional compounds were found to block channel activity by more than 50% at 50 µM concentration when twenty-three analogues of compound 13 were biologically evaluated. These results enabled structure-activity relationship studies and led to the identification of a new series of molecules with a 5-phenyl-2-aminoimidazole core as a new structural class of inhibitors of hH_V1 channels. [5] Moreover, the antiproliferative activity of the seven hits was investigated by testing the compounds in two tumour cell lines, MDA-MB-231 and THP-1, in which H_V1 channels are highly expressed. Most of the molecules were found to be potent growth inhibitors, with compound 13 showing the lowest IC_{50} values (MDA-MB-231 IC_{50} = 9.0 ± 1.0 µM, THP-1 IC_{50} = 8.1 ± 4.3 µM). [5] By bringing together the knowledge and the results from ligand- and structure-based drug design, biophysical and pharmacological characterization, and medicinal chemistry methods, we have obtained promising hits that can be used for further hit-to-lead optimization and serve as chemical tools to better understand the role of H_V1 in tumors and other relevant diseases.

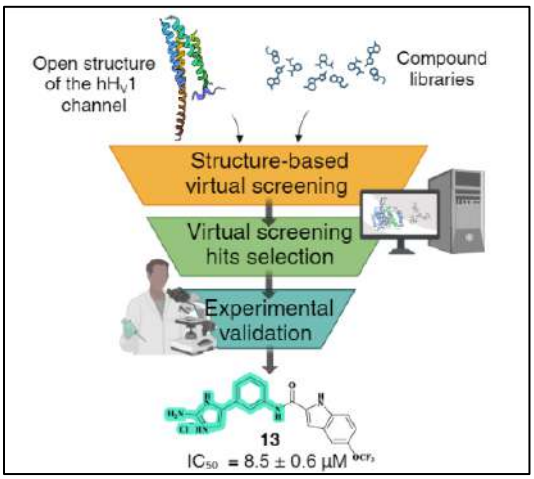

Figure 1: Graphical representation of the research workflow, highlighting the key steps of the study.

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TRIDs therapy for restoring p53 expression on stop mutated cells

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Keywords: cancer, readthrough, mutations, therapy

Stop mutations are genetic alterations characterized by the substitution of a single nucleotide in the coding sequence of a gene, which causes the onset of a premature stop codon (PTC) within the reading frame of the mRNA, resulting in the formation of a truncated and non-functional protein. This type of mutation accounts for approximately 11% of genetic diseases, including conditions such as Cystic Fibrosis, Duchenne Muscular Dystrophy, Choroideremia, Schwachman-Diamond syndrome, and certain types of hereditary cancers involving mutations in the TP53 gene. About 10% of TP53 mutations are stop mutations. [1,2] TP53 mutations create a favorable environment for tumor formation, and mutant p53 may exhibit loss of function, dominant-negative repression, or gain of oncogenic function, contributing to tumor stability and progression. [2] Today there is no therapy for the pathologies caused by this type of mutation, but an approach that has proven to be particularly effective is represented by molecules with readthrough activity (TRIDs; Translational Readthrough Inducing Drugs) which intervene on the ribosome allowing the overcoming of the PTC and the restoration of the synthesis and subsequent functionality of the protein. [3] In this work, we investigate the effects of TRID molecules with readthrough activity on the TP53 gene in tumor cells, which harbors the PTC R213X, the most common TP53 stop mutation that generates a truncated and non-functional p53. We analyzed the restoration of p53 protein expression before and after induction of DNA damage using Western blot, its nuclear localization with fluorescence microscopy, the mRNA expression of p53, and its targets p21 and GADD45 to evaluate the protein's functionality by Real-Time RT-PCR. This study represents a promising path for developing targeted cancer therapies against stop mutations, a new approach to impede tumor proliferation, and a solid foundation for the formulation of novel personalized therapy modalities not only against cancer.

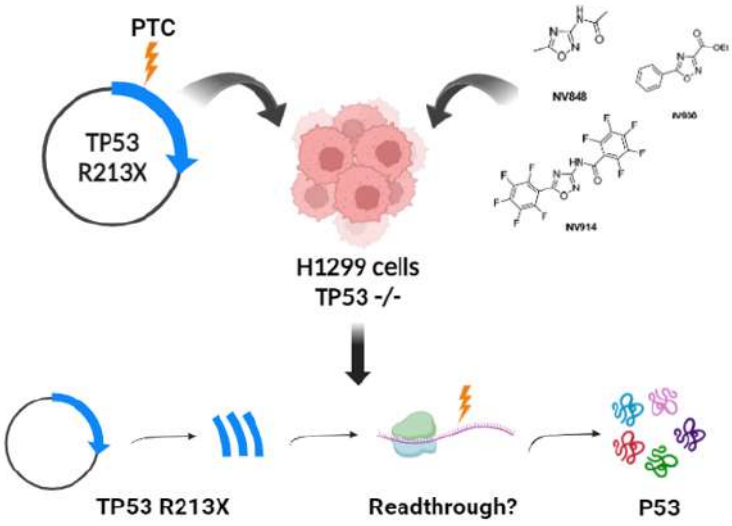

Figure 1: Experimental workflow for the study of the p53 rescue in H1299 $p53^{R213X}$ cells by TRIDs molecule treatment.

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Integrating ligand and structure-based approaches to predict UGT-mediated drug metabolism

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Keywords: drug metabolism, metabolism prediction, machine learning, glucuronidation, UGT

The fate of drugs in the body is governed by their metabolism. For this reason, assessing drug metabolism in the early stages of drug discovery is crucial to understand the stability and safety of novel drug candidates. The increasing availability of metabolic data along with the advancement of machine learning (ML) algorithms in drug design programs has promoted the development of *in silico* tools able to predict the occurrence of a given metabolic reaction and/or the site of metabolism. For this purpose, ligand- (LB) and (SB) approaches can be conveniently integrated to predict, in the first case, the presence and reactivity of specific groups susceptible to the biotransformation and, in the second case, if the molecule fits the enzyme active site and which moiety approaches the catalytic residues. [1] In this work, SB and LB methods were combined to generate Random Forest (RF) classification models for UDP-glucuronosyltransferase (UGT)-mediated metabolism. UGTs catalyze a phase II metabolic reaction consisting of the conjugation of substrates containing nucleophilic sites with a glucuronic acid moiety derived from the cofactor UDP-glucuronic acid (UDPGA). Specifically, our study was focused on UGT2B7 and UGT2B15 isoforms which are involved in the clearance of several drugs as well as in clinically important drug-drug interactions. First, docking and molecular dynamics (MD) simulations were performed to investigate the binding mechanism of cofactor and substrate to UGT2B7 and UGT2B15, exploiting their three-dimensional models available on AlphaFold repository. The analysis of MD trajectories allowed an appropriate conformation of both UGT isoforms to be identified for the development of ML classification models. To this aim, a set of UGT substrates and non-substrates was collected from the manually curated MetaQSAR database [2] and split into training and test sets. SB models were trained on a set of scoring functions computed by docking and rescoring calculations and protein-ligand fingerprints. Instead, the LB classifiers were generated using a set of physicochemical and constitutional descriptors. All models were internally validated by ten-fold cross validation and evaluated on the test set. The resulting models showed satisfactory performances with the LB classifiers outperforming the SB models when evaluated individually. Interestingly, the application of a consensus strategy allowed us to achieve improved performances with respect of the use of the single models highlighting that the two approaches provide complementary information whose integration leads to better results. In addition, the inspection of docking poses furnished useful hints about the structural motifs that are most likely to undergo glucuronidation.

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Electrochemical screen-printed sensor for tacrolimus detection in whole blood: a step toward decentralized therapeutic drug monitoring

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Keywords: Tacrolimus, Therapeutic Drug Monitoring, Electrochemical Sensor, blood analysis.

Tacrolimus (TCM) is a potent immunosuppressant widely used in organ transplant recipients to prevent graft rejection. Due to its narrow therapeutic window and significant inter- and intra-patient variability, Therapeutic Drug Monitoring (TDM) is essential to ensure efficacy while minimizing toxicity. Rapid, accurate, and decentralized methods for TDM are therefore crucial to enable real-time dose adjustments and improve patient outcomes. [1]
Currently, tacrolimus quantification is routinely performed using techniques such as liquid chromatography–mass spectrometry (LC-MS/MS) or immunoassays. [2] While these methods offer high sensitivity and specificity, they require complex instrumentation, specialized personnel, and centralized laboratory infrastructure, which can delay clinical decision-making. In contrast, electrochemical sensors, particularly those based on screen-printed electrodes (SPEs), offer a promising alternative due to their portability, low cost, ease of fabrication, and suitability for point-of-care testing. [3] In this work, we report the development of an electrochemical sensor based on screen-printed technology for the detection of tacrolimus in whole blood samples. The system was optimized using differential pulse voltammetry (DPV) in phosphate buffer (PB) at pH 4.7. Following the optimization of electrochemical parameters, the sensor achieved a detection limit in the low ng/mL range. This level of sensitivity aligns with the therapeutic concentrations typically observed in patient blood samples, making the system suitable for clinical applications. To assess the practical applicability of the sensor, it was employed to analyze real whole blood samples from patients undergoing immunosuppressive therapy. The results were compared with those obtained using conventional analytical methods. A good correlation was observed, highlighting the potential of the sensor for preliminary screening or as a complementary tool in therapeutic drug monitoring (TDM) workflows.
Analyzing whole blood remains inherently challenging due to its complex matrix and the presence of interfering substances. For these reasons, the system will be further optimized and refined to improve performance and specificity. Despite these challenges, this study represents an important step toward the development of portable, rapid, and decentralized tools for therapeutic drug monitoring. The results suggest that electrochemical sensors, when properly optimized and validated, can complement traditional methods and potentially revolutionize the management of immunosuppressive therapies.

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Adipose-Derived Stem Cells and Nutrigenomics: A New Frontier in Obesity Treatment

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Keywords: obesity, nutrigenomics, epigenetics, DNA methylation, adipose-derived stem cells (ADSCs)

Obesity and associated metabolic disorders result from a complex interplay of genetic, epigenetic, and environmental factors, such as diet and lifestyle. Among epigenetic mechanisms, DNA methylation plays a pivotal role in regulating adipogenesis, lipid metabolism, and inflammatory responses. [1] Nutrigenomics has revealed that bioactive dietary compounds can influence the epigenome and subsequently affect both the function and structure of adipose tissue. [2] In mammals, adipose tissue exists in two main forms: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT, characterized by its large unilocular lipid droplet, serves as the primary energy reservoir, while BAT dissipates energy as heat through thermogenesis. [3] Moreover, WAT possesses the remarkable ability to transdifferentiate into beige adipocytes—a process known as browning—which offers promising therapeutic avenues for obesity management. [4,5] The objective of the present study is to evaluate whether natural molecules incorporated into the diet can modulate adipose tissue remodeling via epigenetic mechanisms. In particular, we focus on the potential modulatory effects of fruit-derived nanovesicles (FicoVes) isolated from Opuntia ficus-indica, a Mediterranean plant known for its rich content of bioactive compounds. FicoVes have shown promising anti-inflammatory, antioxidant, and tissue-regenerative properties, [6] suggesting they may serve as effective nutrigenomic agents. Our experimental approach employs human adipose-derived stem cells (ADSCs) and their in vitro differentiated adipocytes obtained from both lean and obese donors. Preliminary viability assays (MTT) indicate that FicoVes, at concentrations of 5, 25, and 50 µg/mL, are non-cytotoxic and do not compromise cell survival. In wound-healing assays, treatment with FicoVes significantly accelerated scratch closure compared to controls, highlighting their potential in promoting tissue repair—a feature that could be highly beneficial in regenerative medicine applications, such as in the treatment of diabetic foot ulcers. Furthermore, Oil-Red-O staining revealed a dose-dependent modulation of lipid accumulation exclusively in mature adipocytes, suggesting that FicoVes exert cell type-specific effects on adipogenesis. In summary, our integrated study demonstrates that, although further epigenetic studies are needed to clarify their precise mechanisms, dietary bioactive molecules such as FicoVes can influence adipose tissue remodeling. These findings underscore the potential role of nutrigenomic interventions in developing novel, minimally invasive therapeutic strategies for the management of obesity and its related metabolic disorders.

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Targeting fat-to-heart crosstalk in obesity: preclinical evidence on the effects of N-acylethanolamines mixture

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Keywords: Acylethanolamides, Cardiometabolic dysfunction, Mitochondrial bioenergetics, High-fat diet (HFD), Adipocyte thermogenesis

Obesity disrupts cardiac function, primarily through excessive fatty acid accumulation and impairs energy metabolism. These changes compromise mitochondrial function, essential for cardiac homeostasis. Adipose tissue contributes to this dysfunction by releasing bioactive molecules and cytokines that promote lipid deposition and inflammation. [1] N-acylethanolamines (NAEs) are endogenous lipids involved in cellular homeostasis, modulating inflammation, pain, food intake, and neuronal function. [1] Here, we evaluated the cardioprotective effects of Olaliamid® (OLA), an olive oil-derived NAE mixture, using in vivo and in vitro models. In vivo, male C57Bl/6J mice (6 weeks old) were fed either a standard chow diet (control) or a high-fat diet (HFD) for 12 weeks. HFD-fed mice received either vehicle or OLA (10 mg/kg/day, per os). At the endpoint, heart tissue was analyzed for lipid metabolism, inflammation, and fibrosis markers via Real-Time PCR and Western blot. In vitro, mature 3T3-L1 adipocytes were pre-treated with individual NAEs (3 μM) or OLA (1–3 μM) for 1 hour before exposure to palmitate (100–200 μM). Mitochondrial bioenergetics was assessed via Seahorse XFe24 analysis, while gene expression of lipid metabolism and mitochondrial markers was evaluated by qPCR. Results showed that OLA increased cardiac AMPK phosphorylation, a cellular energetic sensor, and improved lipid metabolism, reducing Fasn, Cd36, Dgat1, and Pparγ expression. Additionally, OLA reduced inflammation and fibrosis, lowering Il-1β and fibronectin levels. Given the adipose-heart interplay, we explored OLA's effects on adipocyte function. Seahorse analysis revealed that OLA enhanced mitochondrial activity, improving oxygen consumption and ATP production. At the molecular level, OLA upregulated *Ppara*, *Cpt1*, and *Ppargc1a*, promoting lipid metabolism and mitochondrial biogenesis. Notably, OLA induced a white-to-brown adipocyte transition, increasing thermogenic gene expression and lipid storage capacity (PPAR-γ upregulation). Overall, our findings highlight OLA's multitarget effects in restoring fat-to-heart crosstalk disrupted by lipid overnutrition, supporting its potential as a therapeutic approach for obesity-related cardiometabolic syndrome.

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Harnessing hSTING for Enhanced Antiviral response

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Keywords: hSTING, Antiviral, Immune modulation

The human STimulator of INterferon Genes (hSTING) is a key regulator of the innate immune response, crucial for antiviral defense through type I interferon activation. [1] Given its role in infections, cancer, and autoimmunity makes it a promising therapeutic target. [2] The discovery of potent human-STING agonists could revolutionize antiviral and cancer immunotherapy. [3] Based on the structural features of known hSTING agonists and previous work by our group, [4] we have developed a new series of potential STING activators aimed at achieving higher potency and improved pharmacological properties. Our approach integrates rational synthetic design with computational studies to predict the binding mode of these novel compounds and optimize their interactions with the hSTING protein. Following their synthesis and characterization, the biological activity of these compounds has been evaluated to assess their ability to activate STING signaling and induce type I interferon responses, key mechanisms in antiviral immunity. Further investigation on the compounds able to activate hSTING will be carried out to evaluate their ability to inhibit viral replication.

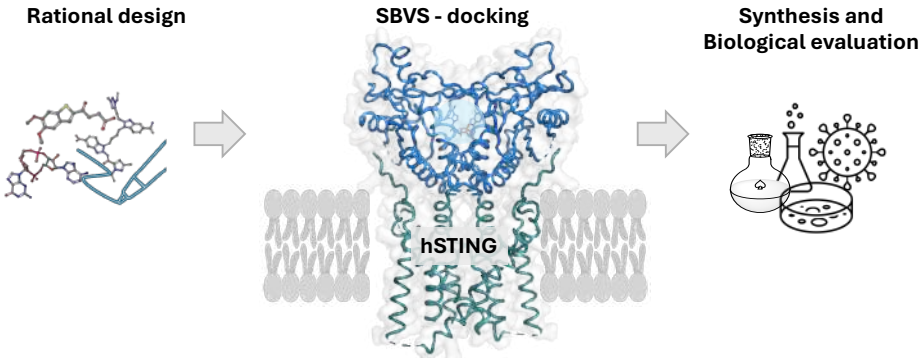

Figure: Project workflow.

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Discovery of the first-in-class pyrazole-oxazole based synergistic antifungal agent: synergy studies, *in silico* investigation, and *in vivo* validation

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Keywords: Candida albicans, chemoresistance, azole drugs, galleria mellonella

Candida albicans is among the most prevalent pathogens responsible for invasive fungal infections (IFIs), thus posing a growing healthcare challenge due to its high morbidity and mortality, particularly in immunocompromised patients. [1] Azole antifungals represent the first-line treatment for both systemic and superficial mycoses, and function by inhibiting ergosterol biosynthesis, a fungal-specific sterol essential for membrane integrity. Of particular note, triazole antifungals like Voriconazole (VRC) are widely used in treating *C. albicans* infections due to their potent inhibition of lanosterol 14α-demethylase (LDM, CYP51), converting lanosterol into ergosterol, and encoded by the ERG11 gene. Despite their efficacy, over years, the abuse and misuse of azoles has led to the emergence of azole-resistant fungal strains, primarily driven by mutations in the ERG11 gene. [2] Notably, C. Albicans strains isolated by patients are resistant to at least one antifungal drug, thus underscoring the urgent need for novel antifungal agents showing efficacy against resistant strains or capable to synergistically enhance the efficacy of existing therapies. Herein, we report the discovery of a *first-in-class* 1,4-pyrazole-isoxazole based compound, namely SL38, which exhibited remarkable synergistic activity when combined with azole drugs. [3] In our study, SL38 was evaluated in combination with VRC against the azole-resistant *C. albicans* strain ATCC 10231. The SL38-VRC combination demonstrated a significant reduction in the yeast-to-hypha transition, a critical virulence factor in *C. albicans*, accompanied by the downregulation of ERG11 gene expression, whereas it is upregulated in response to azole drug treatments, as confirmed by quantitative real-time PCR analysis. To confirm the target engagment, VRC was directly assesset of the isolated LDM enzyme in presence of SL-38, which was able to induce a significant reduction of VRC IC_{50}, in absence of SL38 activity on LDM itself. The synergistic efficacy of the SL38-VRC combination was validated in an *in vivo* model of *Galleria mellonella* infection. Gratifingly, the combination SL38-VRC improved survival rates in infected larvae, whereas VRC alone had no detectable impact, further confirming also in *in vivo* settings the hypotesis of the synergistic effect of this combination. To elucidate the molecular underpinnings of the synergystic effect, in-depth *in silico* studies were performed, unveiling the combination SL38-VRC is able to uniquely occupy all binding sites of LDM. This result may explain the enhanced efficacy of this combination therapy. In conclusion, we identified of a novel promising class of synergistic antifungal agents, which may offer a new glimpse in the battle against azole-resistant *C. albicans* infections.

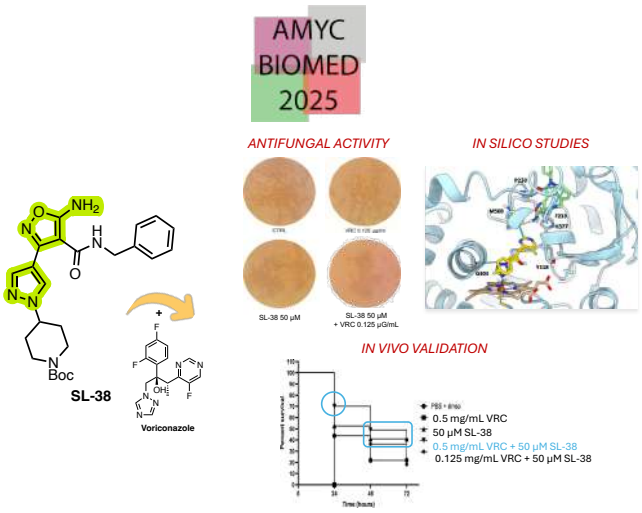

Figure 1: SL-38 synergistic effect in combination with Voriconazole.

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PROTACs: a new strategy to target hDASPO degradation for the modulation of endogenous D-aspartate in schizophrenia

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Keywords: D-Aspartate, D-aspartate oxidase, schizophrenia, degradation, proteolysis targeted chimeras

D-aspartate (D-Asp) is abundant in mammal brain at embryonal level while it rapidly decreases at post-natal stages due to the enhanced activity of D-aspartate oxidase (DASPO). [1] D-Asp participates to glutamatergic neurotransmission and alteration of physiological metabolism is linked to pathologies. Depletion of D-Asp was observed in *post-mortem* brain samples of schizophrenia patients and has been correlated with excessive degradation by DASPO causing the typical reduction of neurotransmission. [2] On the other side, D-Asp administration improves neurotransmission, synaptic plasticity and dendritic length, [3] therefore the modulation of endogenous levels of D-Asp is interesting to develop potential therapies. To this aim, DASPO is a significant target in the absence of evidence for key regulators in the synthesis of D-Asp. As proteolysis targeted chimeras (PROTACs) are a new promising strategy to promote proteasomal degradation of targets of choice, [4] its application on DASPO would promote enzyme degradation to enhance endogenous D-asp levels in brain tissues. PROTACs were synthesised (Figure) based on olanzapine as DASPO-recognising moiety and lenalidomide or (VH032)-Me to recruit Cereblon or Von Hippel Lindau E3 ligases, respectively. Microscale thermophoresis (MST) and DOSY-NMR assays confirmed that our PROTACs bind to their targets forming binary and ternary complexes with K_D in micromolar range. In the near future, mass photometry experiments and crystallisation trials will be performed to better investigate the formation of ternary complexes. Additionally, degradation activity against DASPO will be studied through *in vitro* ubiquitination assays and by glioblastoma and neuroblastoma cell lines treatment.

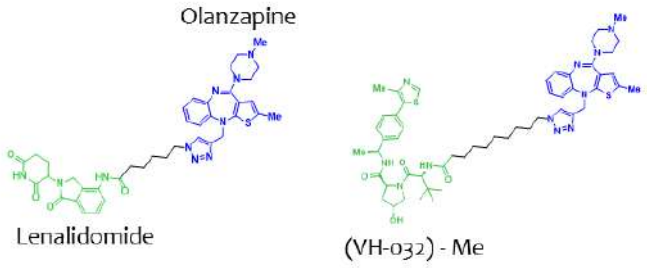

Figure: Design of PROTACs targeting DASPO degradation: Olanzapine (blue) as DASPO recruiter, lenalidomide and VHL ligand (VH-032)-Me (green) as Cereblon and VHL ligase recruiters, respectively. The two moieties are linked by aliphatic carbon chains of different lengths.

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Synthesis of enantiopure (*R*)- and (*S*)-methadone metabolites via a cyclic sulfamidate approach and pharmacological evaluation as novel NMDAR antagonists

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Keywords: Methadone, Metabolites, FLIPR, NMDA uncompetitive antagonists

N-Methyl-D-aspartate receptors (NMDARs) have gained significant attention as a therapeutic target for the development of novel antidepressants. The discovery of the rapid and sustained antidepressant effects of ketamine has led to the approval of two NMDAR uncompetitive antagonists: intranasal esketamine (Spravato®) and oral dextromethorphan-bupropion (Auvelity®). [1,2] These advancements have paved the way for the exploration of additional NMDAR-targeting compounds with improved pharmacological profiles. Among them, esmethadone (REL-1017, (*S*)-methadone) is being developed by Relmada Therapeutics Inc. as a once-daily oral treatment for major depressive disorder (MDD). While both (*R*)- and (*S*)-methadone are NMDAR uncompetitive antagonists, esmethadone is devoid of opioid or dissociative effects. In phase 2 clinical trials, it has shown rapid onset and sustained efficacy, highlighting its potential as a novel antidepressant. [3] Despite the therapeutic promise of esmethadone, the industrial synthesis of methadone presents several challenges. Methadone is commercially produced as a racemic mixture through synthetic routes characterized by poor regioselectivity in a key step, which significantly impacts overall yield. Furthermore, obtaining enantiopure methadones requires complex and time-consuming chiral resolution techniques. [4] To address these limitations, we have developed an optimized and more efficient cyclic sulfamidate-based synthesis of enantiopure (*R*)- and (*S*)-methadone. Additionally, we applied this strategy to design a novel stereodivergent synthetic approach for the asymmetric synthesis of 20 enantiopure human methadone metabolites, whose stereochemistry was assigned by XRD analysis. To explore the pharmacological activity of these compounds, we tested them through fluorometric imaging plate reader (FLIPR) calcium assays to assess their uncompetitive antagonist properties against the 4 more relevant NMDARs subtypes. Furthermore, 10 selected metabolites underwent radioligand displacement assays to evaluate their binding affinity toward a panel of central nervous system (CNS) receptors involved in mood regulation and neuropsychiatric disorders. Our findings reveal a subset of methadone metabolites with significant NMDAR antagonist activity, broadening the understanding of the structure-activity relationship within this class of compounds.
Among methadone metabolites, we identified a set of new NMDAR uncompetitive antagonists, and characterized their pharmacological profile. These compounds could potentially undergo development as fast acting antidepressants or could be developed for other neuropsychiatric conditions and could advance our understanding of the mechanism of action of esmethadone and other uncompetitive NMDARs antagonists.[5]

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AMYC BIOMED 2025

Synthesis of new 5-fluorouracil and heme oxygenase 1 inhibitor mutual prodrugs with antiproliferative effects

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Keywords: Anticancer chemotherapy; mutual prodrugs; 5-fluorouracil; HO-1 inhibitors.

Prodrug strategies have gained significant interest in the challenging field of drug development; particularly, mutual prodrugs are a specialized category in which two or more drugs are covalently linked, either directly or via a cleavable spacer, resulting in a synergistic therapeutic effect that might enhance efficacy while potentially reducing side effects. [1] 5-Fluorouracil (5-FU) is one of the most widely used anticancer drugs, and it is included on the WHO Model Lists of Essential Medicines. This antimetabolite drug is used for the treatment of several solid tumors, including breast cancer and colorectal cancer. However, its short half-life, poor drug-likeness, and high non-specific toxicity significantly limit its clinical use. To counteract these limitations the mutual prodrug approach has been extensively used, and more recently our research group developed novel 5-FU and HO-1 inhibitor (HO-1i) hybrids as an innovative anticancer strategy. [2] Since HO-1 is an inducible enzyme that becomes overexpressed in response to oxidative stress, abnormal HO-1 levels have been linked to cancer progression and resistance to chemotherapy. [3] Therefore, the inhibition of this enzyme is emerging as an innovative pharmacological strategy in adjuvant chemotherapy. In this study, we synthesized three new 5-FU/HO-1i mutual prodrugs by conjugating 5-FU with an arylethanoltriazole derivative (LS 8/66), recently identified for its high inhibitory potency against HO-1 (HO-1 IC50 = 0.53 μM). Derivatives SI 3/02 and SI 3/08 incorporate succinic and glutaric linkers, respectively, while SI 3/07 is a monoester in which the hydroxymethyl-5-FU moiety, used in SI 3/02 and SI 3/08, has been replaced with 5-fluorouracil-1-yl acetic acid (Figure 1). In vitro assessment in PBS, SGF, and human plasma was performed and suggested a suitable chemical and enzymatic stability profile ($t_{1/2}$ = 13-24 hours), while in silico ADMET calculations predicted good druglikeness and pharmacokinetic properties. Finally, newly synthesized compounds were tested to assess their in vitro antiproliferative effects towards selected breast carcinoma cell lines (MCF-7) where the expression level of HO-1 was quantified by western blot analysis. Among the new mutual prodrugs, SI 3/02 showed a significant effect on MCF-7 cells viability better than the reference drug 5-FU, and similar to the combination of corresponding parent drugs, supporting a further in-depth investigation of its promising anticancer effect.

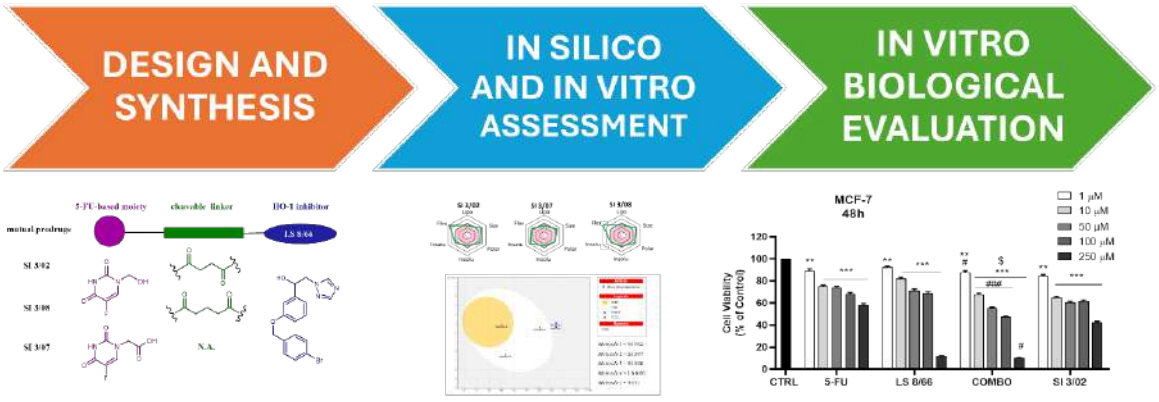

Figure 1: Strategy for the identification of new 5-FU/HO-1i mutual prodrugs.

Acknowledgments
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A Novel Lateral Flow Biosensor for the Early Detection of Ovarian Cancer-Related microRNAs

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Keywords: Lateral Flow assay, microRNA, NALFA

Ovarian cancer (OC) remains one of the most aggressive gynecological malignancies, [1] characterized by high mortality rates due to its asymptomatic progression in early stages [2,3] and the lack of sensitive, non-invasive diagnostic tools. While current clinical approaches rely on imaging techniques and CA-125 biomarker detection, these methods present limitations in terms of specificity, sensitivity, and accessibility, making them unsuitable for early-stage screening. [4] Consequently, the search for alternative biomarkers and novel diagnostic strategies is a priority in oncology research.Emerging evidence highlights the role of microRNAs (miRNAs) as promising biomarkers for OC detection. These small, non-coding RNA molecules (18-25 nucleotides) exhibit dysregulated expression patterns in cancerous tissues and are released into the bloodstream, making them valuable indicators of disease onset. [5] However, traditional miRNA detection techniques—such as qRT-PCR, microarrays, and sequencing—are labor-intensive, costly, and impractical for large-scale applications, emphasizing the need for innovative, rapid, and portable diagnostic solutions. In this study, we present a lateral flow assay (LFA)-based biosensor designed for the detection and quantification of OC-associated miRNAs in capillary blood samples. The proposed system integrates nucleic acid lateral flow assay (NALFA) principles, combining gold nanoparticles (AuNPs) for visual readout with horseradish peroxidase (HRP)-mediated chemiluminescent (CL) amplification to enhance sensitivity. Specifically, the biosensor utilizes a dual functionalization approach, where AuNPs are covalently modified with thiolated DNA probes and simultaneously adsorb HRP (AuNPs@HRP@DNA_probe). This configuration enables signal amplification, allowing quantitative measurement of miRNA concentrations. The detection mechanism is based on a sandwich hybridization format, where target miRNAs bind to capture probes immobilized on a nitrocellulose membrane (test line) and are subsequently detected through hybridization with the AuNPs@HRP@DNA_probe conjugate, producing both plasmonic and chemiluminescent signals.

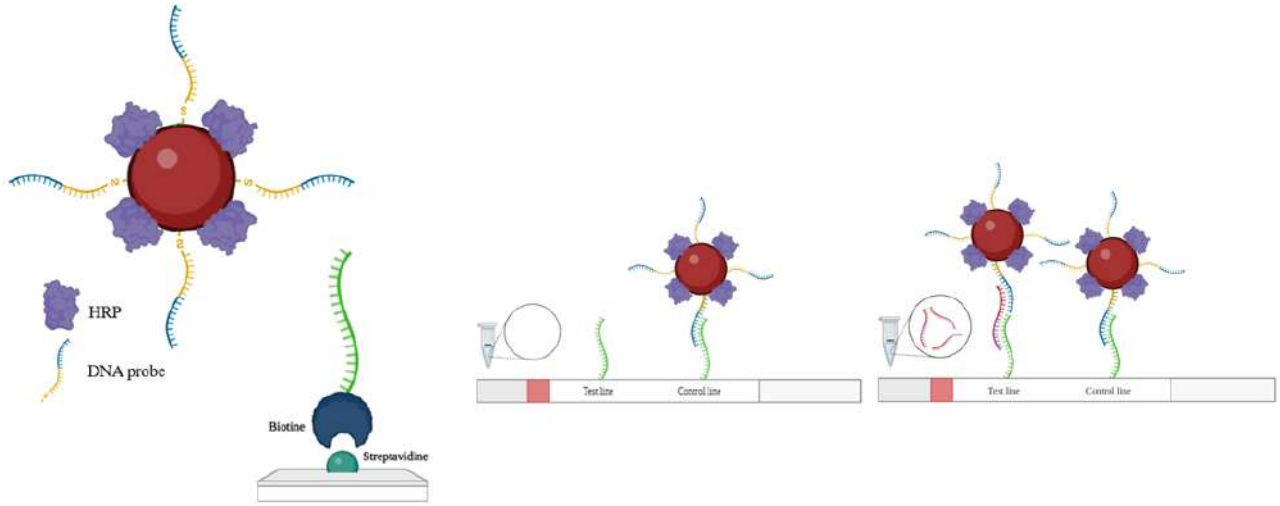

Figure: Schematic representation of DNA/HRP based lateral flow assay for microRNA detection

By leveraging the simplicity, portability, and cost-effectiveness of the LFA platform, this biosensor represents a promising tool for early OC diagnosis. Its user-friendly nature, coupled with smartphone-based signal quantification, enables decentralized testing, making it particularly suitable for large-scale screenings and point-of-care applications, especially in low-resource settings. This innovation holds the potential to facilitate earlier cancer detection in order to improve clinical decision-making.

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Design and synthesis of a new generation of pan-RNA-virus inhibitors

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Keywords: Antiviral, emerging viruses, ssRNA viruses

The recent pandemic caused by SARS-CoV-2 has shown to the entire world that a single virus could cause epidemics that can evolve into pandemics. New pathogens emerge and spread in human populations due to several factors, including high population density, urbanization, deforestation, climate change, increasing human-wildlife interactions, increased international travel, migratory flows, health system breakdowns, social injustices, and geopolitical conflicts. Among the emerging viruses, ranked as high-risk, we can list SARS-CoV-2, Zika virus and Usutu. [1,2] While vaccines remain crucial in controlling infectious diseases, the rapid emergence of novel viruses with pandemic potential highlights the urgent need for effective pharmacological treatments. The Panviride project, supported by the INF-ACT foundation through PNRR funding, aims to develop innovative therapeutic strategies to limit the spread of emerging viruses and treat infectious diseases lacking effective therapies. [3] We selected, from an in-house library of small molecules, the compounds with reported antiviral activity, specifically, against ssRNA viruses. [4,5] The compounds that previously showed the best antiviral activity were re-synthesised and assayed against SARS-CoV-2, Zika virus and Usutu showing promising results. Compounds were clustered per SARs and the most pan-active compounds were selected for further investigation of the mechanism of action. Compound PS1097 showed the highest potency against the three studied viruses and 4 more analysed viruses, all sharing the replication localization in the endoplasmic reticulum, suggesting a host-target mechanism of action for PS1097. It was further confirmed the interference of PS1097 with the ER membrane remodelling, used by these viruses for replication.
The most potent compounds have been selected as Hit compounds for the design and synthesis of a second generation (Figure 1) with potential antiviral activity. Derivatives belonging to this generation will be tested against the three viruses above mentioned. Here we report the (re)synthesis of the two generations of compounds, the antiviral efficacy and the cytotoxicity and planned follow up for these hit compounds.

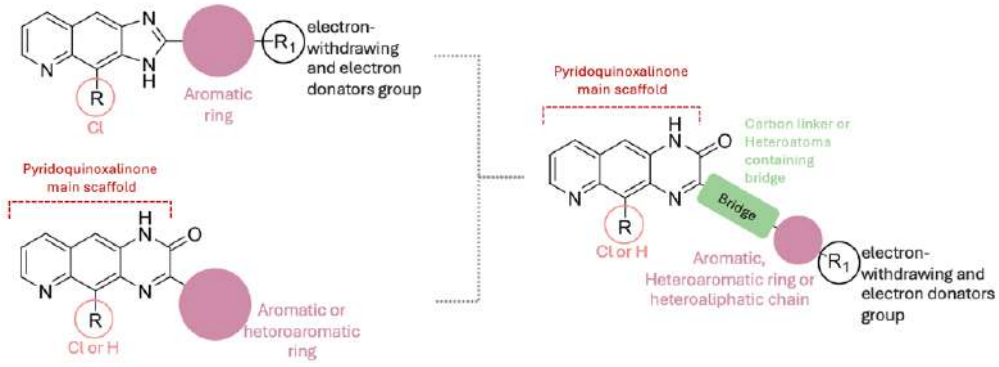

Figure 1: Design of the second generation

Acknowledgments

This research was supported by 'Bandi a cascata' nell'ambito del PNRR, Misura 4 Componente 2 Investimento 1.3 – Programma di ricerca e innovazione PE00000007 "One Health Basic and Translational Research Actions Addressing Unmet Needs on Emerging Infectious Diseases (INF-ACT)", finanziato dall'Unione europea – NextGenerationEU; Progetto finanziato: Preparedness Against Newly- emerging Viruses: Innovations in Research, Intervention, and Drug Evolution (PANVIRIDE) contrassegnato dal codice identificativo S5.P0002.

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Circulating miRNA-21 monitoring in Triple Negative Breast Cancer: A Step Toward Portable Liquid Biopsy

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Keywords: Screen-printed electrodes; TNBC; miRNA; Liquid biopsy, Point-of-care

Triple-negative breast cancer (TNBC), accounting for approximately 15–20% of all breast cancer cases, is a highly heterogeneous and aggressive subtype correlated to rapid progression, elevated metastatic potential, and poor clinical outcomes. Given the complexity and variability of TNBC, along with its limited therapeutic options, early and accurate diagnosis becomes critical for cancer prognosis improvement and timely therapeutic decisions. In this context, liquid biopsy has emerged as a promising solution—enabling minimally invasive cancer diagnosis and monitoring through the detection of related biomarkers in bodily fluids. Among these, microRNAs (miRNAs), that are single-stranded non-coding RNAs, typically 17–27 nucleotides in length, play key roles in gene regulation. They emerge as a valuable choice due to their stability and ability to reflect the tumor's heterogeneity. [1] Several studies have consistently reported miRNA-21 up-regulation in TNBC, associating it with poor prognosis and confirming its potential for early diagnosis, risk stratification, and clinical treatment. [2] Traditional approaches, even if they are highly accurate, they can be labor-intensive, costly, and demand highly skilled personnel; therefore, limiting their implementation in low-resource or decentralized health care settings. As a result, the demand for detection platforms that are rapid, affordable, and user-friendly is emerging. These challenges can be addressed by electrochemical point-of-care (POC) platforms, inspired by the glucose strip, offering a good alternative by reducing matrix effects, integrating cost-effective and eco-friendly substrates. To this context, we developed a portable and cost-effective miRNA-21 detection platform for TBNC monitoring. [3] The miRNA-21 target was selectively detected using a complementary DNA probe modified with methylene blue, as a redox mediator, immobilized onto an AuNPs-functionalized, paper-based screen-printed electrode. Significant experimental parameters and sensor's selectivity were carefully optimized and evaluated, allowing miRNA-21 detection in both standard solution and human serum. The proposed biosensing platformed achieved limit of detection of 1.2 nM, high miRNA-21 specificity and satisfactory repeatability of about 8%. The platform's performance was improved 10-fold with the integration of an external paper-based origami pre-concentration device, enabling pM-level miRNA detection and advancing its potential for real clinical practice applications. The platform is envisioned as a starting point for developing accessible, rapid, cost-effective POC testing, with significant implications for personalized medicine and early TNBC detection.

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siRNA Therapeutics and the Hurdles of Delivery: Insights from ANANAS Nanoparticles

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Keywords: Drug delivery, Nanoparticles, siRNA, Targeting, Cancer.

The elucidation of the RNA interference (RNAi) mechanism sparked significant interest in the development of gene-targeting methodologies based on RNA oligonucleotides. Small interfering RNAs (siRNAs) are double-stranded, non-coding RNA molecules capable of post-transcriptional gene regulation by specifically silencing targeted messenger RNAs (mRNAs). [1] Their versatile design, low effective concentrations, and potential for treatment with minimal side effects make them powerful therapeutic tools. [2] In the field of cancer, the use of siRNAs holds promise for down-regulating mutated genes and suppressing unwanted gene expression, offering a strategy to inhibit tumor cell growth and invasion [3] Despite their potential, the use of siRNAs in therapy present various challenges mainly due to their considerable size (21-23 nucleotides, 13-15kDa), negative charge, vulnerability to ribonucleases and difficulties in achieving precise tissue targeting1. To address these issues, suitable carriers are required to facilitate siRNA delivery, ensuring protection from serum nucleases, targeted cellular delivery and successful endosomal escape. [1] We propose utilizing ANANAS nanoparticles (Avidin Nucleic Acid Nano Assemblies) as a siRNA delivery system. ANANAS nanoparticles are colloidally stable, nanosized polyavidin toroids (approximately 120nm in size, containing about 350 avidins per nanoparticle) that exploit the high-affinity interaction between avidin and biotin (with a Kd of approximately 10^{-15}) to bind up to 1000 biotinylated elements per nanoparticle. [4] ANANAS nanoparticles offer a versatile platform for creating multifunctional carriers. This high-affinity targeting system is envisioned to (a) protect siRNA from RNase degradation, owing to ANANAS PEGylation and the avidin/siRNA-biotin system, (b) deliver intact siRNA to the cytoplasm of living cells through lysosomal escape strategies and S-S bond cleavage between biotin elements and siRNA in the cytosol, and (c) selectively target (e.g., antibodies or peptides) cells and tissues of interest. Despite successfully constructing and characterizing the multifunctional platform, our system did not achieve gene silencing for the selected targets (GFP and VDAC-1). In response to this outcome, we systematically investigated possible limiting factors, leading us to modify the siRNA release strategy using an acid-cleavable linker, explore alternative lysosomal escape enhancers (e.g., lipid-like molecules such as FTY-720, CADs including nortriptyline and loperamide, and fusogenic peptides), and improve siRNA stability through chemical modifications and condensing agents. Despite extensive optimization, gene silencing remains elusive. This study highlights the complexities associated with siRNA delivery: encountering obstacles is an inherent part of the process, and through continued refinement, we aim to advance the development of effective siRNA-based therapeutics.

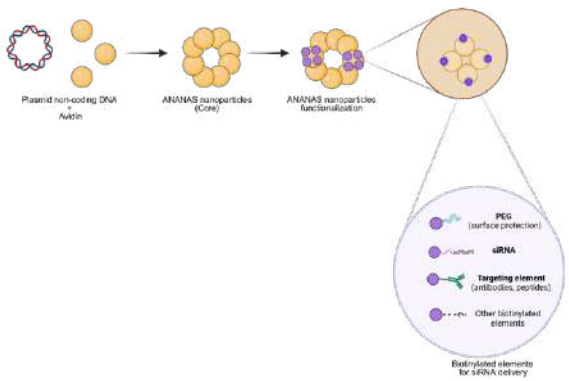

Figure 1: ANANAS nanoparticles composition and biotinylated elements for siRNA delivery.

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Interaction of mRNA-based COVID-19 vaccines with lung adenocarcinoma cells

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Keywords: Solid Lipid Nanoparticles, mRNA-based vaccines, A549 cells, Epithelial-Mesenchymal Transition, Spike Protein

The anti-Covid vaccination campaign has changed the perception of the scientific community on the use of nanometric systems for the delivery and release of nucleic acids. However, in order not to frustrate the transition from hope to skepticism, it is necessary to assess the bio-nano interaction at the level of the single cell. In the present study, we used solid lipid nanoparticles (SLNs) carrying the mRNA encoding the Spike protein of SARS-CoV2 (Comirnaty). After an evaluation of the chemical and physical properties, SLNs were made fluorescent with a dye stably internalized and not modifying either the shell or the mRNA. Imaging studies, performed on human lung adenocarcinoma A549 cells, confirmed that SLNs: a) penetrate rapidly through an endocytosis process; b) exploit a vesicular transport system; c) are able to escape from the endosomes and to avoid further entrapment in lysosomes. [1] The ability to release a functional mRNA is confirmed by molecular biology studies and ELISA assays that highlighted an early translation of the Spike protein and a release into the supernatant. Cytotoxicity studies have shown a dose-dependent reduction of the survival of A549 cells starting from 0.6 μg/ml. Since A549 cells express the human receptor for angiotensin converting enzyme 2 (hACE2), this effect could be ascribed to an interaction between the produced Spike and the receptor. However, the evidence of a similar toxic effect on cells not expressing hACE2 after exposure to Comirnaty, lead us to exclude the above-mentioned hypothesis. Interestingly, a specific phenotypic modification occurred in A549 cells from 24 to 96 hours of treatment. More specifically, a high percentage of cells lost the epithelial pattern with strong adhesion and well-spread cytoplasm showing a polarized structure and an elongated shape. This modification is typical of epithelial mesenchymal transition (EMT) observed in lung epithelial or carcinoma cells under specific stimuli. The morphological alteration was supported by the analysis of three targets, α-SMA, vimentin and E-cadherin, which changed their expression in line with the EMT process. Finally, the treatment with transforming growth factor-β (TGF-β), a typical EMT trigger, induced morphological alterations similar to those found upon the Comirnaty incubation in A549 cells. The same experiments with Comirnaty carried out in cells able to go through EMT but not expressing the hACE2 receptor did not lead to any morphological modification. To better understand the role of Spike expression on the induction of EMT, experiments in A549 cells exposed to Comirnaty, using antibody binding the released Spike or hACE2 receptor, are in progress, whereas other controls, such as empty-SLNs and free mRNAs will be included. Further investigations need to be done, but these results better clarify the stability of mRNA-based vaccines and their interaction with cells, leading to an ever-conscious development of nanovaccines.

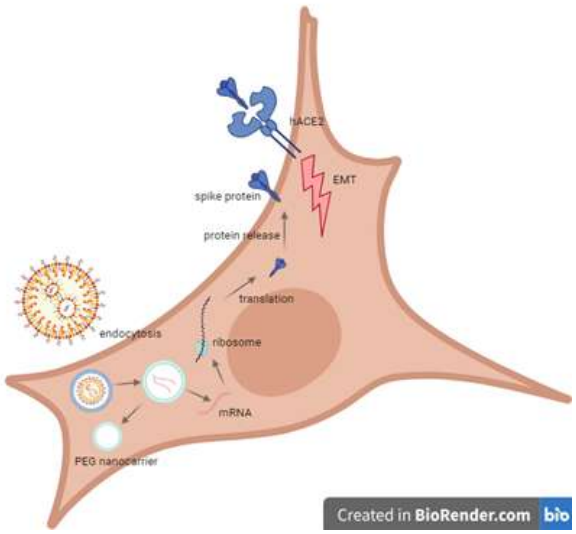

Figure: mRNA-SLNPs vaccines' endocytosis pathway into target cells. Once internalized, the nanocarrier releases the cargo mRNA into the cytoplasm, where it can be translated into Spike protein. The protein exposure at the membrane level or exocytosis then occurs. The hACE2 receptors, present on A549 cells, are then able to interact with the released Spike proteins and potentially trigger some intracellular pathways.

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Anticancer potential of diiron bis-cyclopentadienyl complexes

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Keywords: dinuclear complexes, medicinal chemistry, iron, cancer

Nowadays, a few platinum compounds are used worldwide in the hospital treatment of different types of tumours. However, despite their efficacy, these drugs lead to severe side effects. [1] Compounds containing endogenous metals have been considered as an alternative to overcome these issues. [2] Specifically, iron is non-toxic and abundant. Recent studies have unveiled the anticancer potential of dinuclear iron(I) complexes based on the bimetallic Fe_2Cp_2 core (Cp = η5-C_5H_5), [3] that enables cooperative effects offering wide synthetic opportunities. In particular, mono and bis cationic stable complexes with hydrocarbyl bridging ligands such as aminocarbyne (Fig.1 , Structures 1 and 2) and vinyliminium (Fig.1, Structure 3) can be easily obtained. [4,5] In vitro and, in selected cases, in vivo studies have been performed on these diiron complexes, suggesting an activity linked to the intracellular disaggregation of the diiron structure, triggering reactive oxygen species production and inhibition of thioredoxin reductase. [5,6] An overview of the most salient findings will be presented, covering both synthetical and biological aspects.

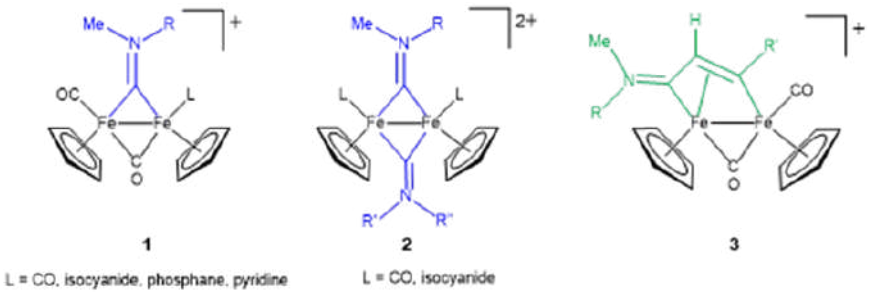

Figure 1: Structures of diiron complexes with bridging hydrocarbyl ligands: mono-aminocarbyne complexes (1), bis-aminocarbyne complexes (2), vinyliminium complexes (3). R/R'/R'' = alkyl or aryl substituents.

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AMYC BIOMED 2025

Shaping ZnO Synthesis through Design of Experiments to Enhance Photocatalysis and Antibacterial Activity

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Keywords: Zinc oxide (ZnO); Design of Experiments (DoE); Photocatalytic activity; Micro/nano-robots (MNRs); Antibacterial applications.

The widespread misuse of antibiotics over the past decade has led to the emergence of antibiotic-resistant bacterial strains, complicating infection treatments. Along with the synthesis of new antibiotics, materials-based strategies could be designed to tackle antibiotic resistance. For instance, Zinc oxide (ZnO) when in the form of nanostructured material shows promise for photocatalytic and antibacterial applications, due to the release of reactive oxygen species and zinc ions. However, ZnO nanoparticles are hindered by poor colloidal stability and toxicity to various cell lines. To address such issue, this study employs a Design of Experiments (DoE) approach based on a two-level factorial design to assess the effects of Zn precursor concentration, KCl concentration, and reaction time on the ZnO's functional properties. The synthesised ZnO materials were analyzed via SEM, XRD and UV-visible reflectance. Photocatalytic performance was tested by methylene blue dye degradation under simulated solar light. Building upon an optimized synthesis for wearable electronics, [1] the DoE approach identifies the optimal synthesis conditions whilst minimising time and costs. Unlike prior studies that overlooked parameter interactions [2] or lacked response surface analysis, [3] this study integrates the influence of Zn precursor concentration, KCl concentration and reaction time on reaction yield and photocatalytic activity. Moreover, based on these parameters, clusters of optimal conditions were identified through Principal Component Analysis (PCA), pinpointing the best-performing samples (Fig.1). Additionally, cellulose-based composites, such as ethylcellulose, APTES-cellulose, and microcrystalline cellulose, were studied as structural supports, complementing prior studies on ZnO/cellulose acetate composites. [4] These composites provide stable platforms for ZnO's biomedical applications, with preliminary results confirming antibacterial efficacy against Gram-positive and Gram-negative bacteria. The DoE-driven approach and KCl-induced (002) plane stabilization establish a framework for improving ZnO's photocatalytic and antibacterial properties. Modified synthesis protocols are under development to achieve asymmetric functionalization with the aim of enabling micron-scale motion in micro-nano robots (MNRs). These findings open avenues for further studies on silver- or copper-based doping, enzyme functionalisation, and cellulose-based composites, aiming to refine ZnO's asymmetric properties. Such progress has potential implications for antibacterial technologies and innovative MNR designs.

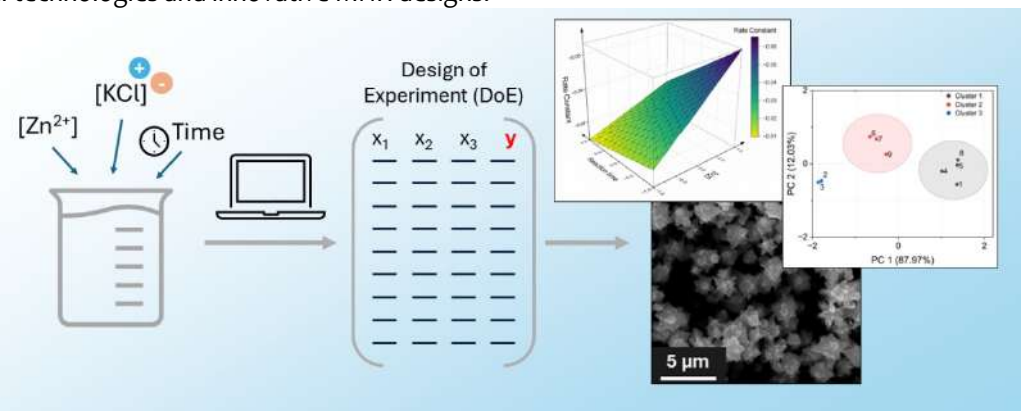

Figure 1: Graphical abstract.

Acknowledgments

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Zinc-aided Mechanism of Splicing Covalent Inhibitor

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Keywords: pre-mRNA Spicing, drug design, Spliceostatin A, computational biochemistry, bioinorganic reaction

Pre-messenger RNA (pre-mRNA) splicing is a crucial process in gene expression, involving the excision of non-coding introns and the ligation of coding exons to produce mature mRNA. [1] Pharmacological modulation of this process has emerged as a promising strategy in cancer therapy, with several small molecules shown to restore splicing fidelity and reprogram gene expression profiles. [2] Among these, members of the spliceostatin family are potent splicing modulators; notably, Spliceostatin A (SSA) exerts its anticancer effect by covalently binding to the SF3b complex via a mechanism that has remained unclear. [3] Using multiscale all-atom simulations, we elucidated the molecular details of SSA's covalent interaction with PHF5A, a zinc finger protein within the SF3b complex. Our results reveal that SSA undergoes a metal-assisted, enzymatic-like reaction, in which the zinc ion activates the thiol group of Cys26 for nucleophilic attack. Simultaneously, a nearby Lys-Asp residue pair facilitates electrophilic activation of SSA's epoxide warhead, enhancing its reactivity and enabling covalent bond formation. Additionally, bioinformatic analysis identified structurally similar zinc finger motifs containing reactive cysteines in other spliceosomal components, suggesting a broader applicability for covalent modulation strategies. These findings offer mechanistic insight into SSA's unique mode of action and lay the groundwork for the rational design of novel covalent splicing modulators targeting RNA-binding proteins involved in pre-mRNA processing and gene regulation.

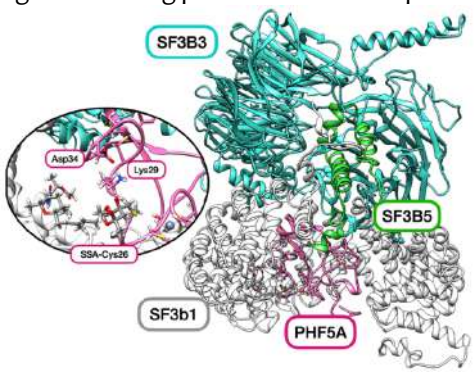

Figure 2: Components of the SF3b complex. The inset shows a close up on the SSA binding site with the relevant residues involved in the covalent linkage reaction mechanism

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Rationalizing the structural basis of organic-platinum hybrid complexes binding towards quadruplex-duplex hybrids through all-atom simulations

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Keywords: Quadruplex-Duplex Hybrids, Molecular Dynamics, Platinum Complexes, DNA-binding

Guanine-rich sequences with complementary base pairs can adopt non-canonical quadruplex-duplex hybrid (QDH) conformations. [1] These structures differ markedly from both canonical double helices and G-quadruplexes, resulting in the formation of a unique binding pocket. Recently, two organic-metal hybrid platinum complexes have demonstrated strong and selective binding towards a particular type of QDH featuring a lateral stem-loop duplex. [2] However, the solution structures of all investigated compounds remain undetermined, leaving open key questions about the structural features that influence their different binding affinities. In this study, to bridge this gap, we conducted all-atom molecular dynamics simulations to explore the mechanisms driving the high selectivity of these compounds at atomic level. Our findings reveal that binding selectivity arises from a combination of extended π–π stacking interactions within the G4-duplex interface and the formation of stable hydrogen bonds with adjacent nucleobases. These insights can guide the rational development of compounds specifically targeting QDH structures.

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Hydrophilic no3py derivative for Cu(II) coordination in nuclear medicine

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Keywords: Nuclear Medicine, Copper, Theranostic, no3py, Lipophilicity

Copper (II) plays an important role in nuclear medicine. In fact, it shows three different isotopes that can be exploited to perform theranostic applications: ^{61}Cu ($T_{1/2}$: 3.33 h), ^{64}Cu ($T_{1/2}$: 12.7 h) and ^{67}Cu ($T_{1/2}$: 61.83 h). However, to exploit a radiometal in a biological environment, it requires a chelating system that forms a stable and inert complex, coupled with a suitable targeting vector. Polyazamacrocycles represent a fundamental family of chelators for a large variety of metals and applications. Some of these are known to form stable complexes with Copper (II) such as NOTA, no3py or cyclam derivatives. In particular, no3py forms stable and inert complex with high selectivity for Copper (II) surprisingly quickly at room temperature. [1] Chelating systems are normally linked to bio-vectors such as antibodies or peptides who can bring the desired radiation into the specific biological compartment (usually tumoral cells). [2] Peptides have considerably smaller size compared with antibodies. For this reason, the impact of the chelating system of choice on the molecular properties of the final bioconjugate cannot be ignored. It is well known that the charge of a molecule and its lipophilicity strongly influences its biodistribution as well as its pharmacokinetic and pharmacodynamic profiles. [3]
For this reason, we decided to use a no3py derivative containing non coordinating sulfonic moieties to coordinate copper (II). [4] This would add three negative charges to the ligand, resulting in a mono negatively charged complex with Copper (II), and an increased hydrophilicity.
In this study, the influence of sulfonic groups on the ligand and the corresponding properties of the copper (II) complex were investigated. Particular attention was given to the coordination capabilities, redox behavior, lipophilicity, and charge distribution within the structure.

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Cuproptosis: a new frontier in targeting PDAC cells

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Keywords: PDAC, cuproptosis, cancer stem cells

Pancreatic cancer, with pancreatic ductal adenocarcinoma (PDAC), accounting for more than 80% of new cases, is an extremely aggressive malignancy affecting the digestive tract and remains the deadliest form of cancer in humans, with a 5-year survival rate as low as 13%. By 2025, PDAC is projected to become the third leading cause of cancer-related death in Europe. Conventional therapies fail to eradicate PDAC due to its heterogeneity, a dense stromal environment, and the presence of cancer stem cells (CSCs). CSCs play a key role in tumorigenesis, chemoresistance, metastasis and relapse. Notably, CSCs have distinct metabolic profiles, particularly a strong reliance on mitochondrial metabolism, making them highly susceptible to cuproptosis – a recent discovered form of copper-dependent cell death. Cuproptosis occurs when intracellular copper accumulation triggers mitochondrial dysfunction, protein aggregation, and ultimately, cell death. [1–4] Interestingly, our proteomics data already obtained on parental and stem-like PDAC cell lines indicate that CSCs exhibit an upregulation of *DLD, DLAT, DLST, PDHB* and *PDHA1,* subunits of mitochondrial enzymatic complexes recognized as target for cuproptosis. Based on these preliminary proteomic data, and with the aim of developing new therapeutic strategies against PDAC, we hypothesized that CSCs could be eradicated through cuproptosis. The aim of this study was to evaluate new copper ionophores that may promote cuproptosis in PDAC cells by a multidisciplinary approach combining analytical chemistry techniques (spectrometry, chemometrics) with synthetic chemistry, cell biology, and biochemistry. First of all, we focused on the 8-aminoquinoline (8AQ), checking its capability to form a complex with copper by UV-Vis titration and ESI-MS analysis. Preliminary *in vitro* testing on human lung cancer cells (A549) both in absence and in presence of copper along with the study of the interaction of 8AQ with glutathione (γ-glutamyl-cysteinyl-glycine, GSH), a central regulator of redox homeostasis in animal cells, pointed out that 8AQ facilitates the transport of copper into the cells, where the metal is promptly released upon interaction with GSH. This mechanism agrees with that observed for known ionophores featuring scaffolds based on the 8HQ moiety. [5] In light of these promising results, *in vitro* tests on the Panc-1 cell line were conducted to examine the effect of CuCl2 on cell viability, both alone and in combination with varying 8AQ concentrations over different time points (24, 48, and 72 hours). The IC_{50} value was calculated, showing that 8AQ can kill PDAC cells at micromolar concentrations. Next, Elesclomol, a copper ionophore recognized as a potent anticancer drug and evaluated in numerous clinical trials, [6] was tested on PDAC cells in combination with $CuCl_2$ at different concentrations and time points. The obtained data showed that Elesclomol and 8AQ have comparable IC_{50} values. Immunoblotting assays are ongoing to confirm the induction of cuproptosis. Our future perspective is to carry out additional *in vitro* studies using different PDAC cell lines, performing proteomics analysis and encapsulating the most effective copper ionophore so to yield nanoparticles for subsequent *in vivo* studies.

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Effect of a Postbiotic-Enriched Diet on the Antioxidant Capacity of Mytilus galloprovincialis Exposed to Cu2O

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Keywords: Copper, oxidative stress, mussels, postbiotics, antioxidant activity

The aim of this study is to determine the effects of Cu2O exposure on the gills of Mytilus galloprovincialis and the potential protection of a diet that is rich in postbiotics derived from kefir microorganisms. The biological advantages of postbiotics, which consist of microbial cell components and metabolic byproducts such as proteins, lipopolysaccharides and short-chain fatty acids, are largely recognized, with a focus on modulating immunity and enhancing antioxidant defenses. [1, 2] Mytilus galloprovincialis is a bioindicator species because it is a filter-feeding species and is highly sensitive to pollutants like copper, which can lead to oxidative stress by producing reactive oxygen species (ROS) [3, 4]. In response, mussels activate key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST). Long-term exposure to Cu2O can lead to cellular damage, apoptosis and immune dysregulation[5-7].
In the study mussels were separated into two experimental groups, one fed a standard algal diet (G1) and one fed a postbiotic-supplemented diet (G2), both exposed and unexposed to Cu2O.
The results indicate that postbiotic supplementation significantly enhanced the antioxidant response. Specifically, mussels in the G2+ group (postbiotic-fed and Cu2O-exposed) exhibited increased CAT and GST activity compared to the Cu2O-exposed control group (G1+), suggesting a protective role of postbiotics against oxidative stress.
Additionally, the expression of heat shock proteins (HSP70 and HSP90), markers of cellular stress, was lower in postbiotic-fed mussels exposed to Cu2O, indicating reduced cellular damage. Total hemoglobin count (THC) and cell viability analyses showed that postbiotic supplementation regulates immune responses, stopping excessive immune activation and preserving cellular integrity.
This study highlights the significance of postbiotics as functional feed additives in mussel aquaculture, providing a novel approach to enhance resilience against environmental pollutants. The use of postbiotics could contribute to sustainable aquaculture practices, improving the health and physiological performance of mussels while mitigating the impact of heavy metal contamination on marine ecosystems.

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Rational Design of 3D-Printed Microfluidic Columns for Integration with Chromatographic Systems

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Keywords: Chromatography, Microfluidics, Rationalization, 3D Printing

The emerging field of 3D-printed microfluidic tools for biopharmaceutical purification, particularly in chromatography, offers significant opportunities to streamline production processes, enhance efficiency, and reduce material consumption. [1-3] Recent advances in 3D printing technologies have enabled the access to rapid prototyping and fine-tuning of chromatographic stationary phases, in the direction of optimized surface area-to-volume ratio and enhanced control over mass transfer, [4-6]. Despite these benefits, challenges persist, particularly regarding the integration of 3D-printed devices with existing automated systems. The standardization of 3D-printed tools is essential, as it ensures consistency, reproducibility, and compatibility with established systems. [7] Without standardization, the performance may be inconsistent, hindering their broader adoption into existing workflows and industrial application. This work focuses on developing 3D-printed microfluidic chromatography columns compatible with current biopharmaceutical purification systems, while providing high-quality and resource-effective data for process digitalization. By applying Quality-by-Design protocols, the research aims to standardize their production. Initially, the design focused on the external shell of the microfluidic columns, utilizing commercially available materials for prototyping. A key aspect of this study was identifying critical design parameters to optimize the column performance. To guide this process, graphical methods, also from published literature, [8] were used to select materials and develop column geometries that would enhance mechanical strength and flow uniformity. A microfluidic column was successfully printed, the design reported in Figure 1, and characterized for packing control and chromatographic efficiency. Its results were compared to standard commercial columns, demonstrating potential for integration with established systems such as HPLC and preparative LC devices. In addition, the study leveraged computational fluid dynamics simulations to design stationary phase, predicting the behavior of various channel geometries. The simulations showcased that square-shaped channels offered the best flow homogeneity, making them ideal for high-resolution separations, while other geometries, such as triangular channels, may offer benefits for specific applications requiring faster separations. In conclusion, this work lays the groundwork for integrating 3D-printed microfluidic chromatography columns into biopharmaceutical purification workflows, highlighting their potential for customization to meet specific needs. By optimizing design and ensuring compatibility with commercial systems, the 3D-printed chromatography tools could be broadly adopted, enabling more cost-effective and tailored purification processes.

Figure: The design of the microfluidic column

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Engineering a High-Affinity Fn3 Scaffold Protein for Selective Targeting of Mesothelin in Oncology

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Keywords: mesothelin, p**rotein-protein docking, molecular dynamics, deep learning**

Mesothelin (MSLN) is a cell surface glycoprotein that is overexpressed in various solid tumors, including mesothelioma, ovarian, pancreatic, and lung cancers. [1] By interacting with the cancer-associated antigen mucin 16 (MUC16), MSLN promotes tumor cell adhesion and metastasis. Due to its limited expression in normal tissues, MSLN has emerged as an attractive therapeutic target. [2] Nonetheless, antibody-based therapies showed limited clinical efficacy because of off-target effects and reduced specificity. To address these limitations, we developed Fn3_5, a high-affinity binder derived from the 10th type III domain of human fibronectin (Fn3). We employed a combination of protein-protein docking, deep learning-based structural prediction, molecular dynamics (MD) simulations, and MM-PBSA free energy calculations to predict and model the interaction between Fn3_5 and MSLN. The predicted binding site is located on the N-terminal region of MSLN, overlapping with the MUC16-binding interface, suggesting that Fn3_5 may competitively prevent MUC16 interaction. Epitope mapping using yeast surface display confirmed preferential binding to MSLN domain B, with enhanced affinity in the presence of domain C. MD simulations revealed stable and well-defined interactions, characterized by persistent H-bonds, electrostatic complementarity, and aromatic π–π stacking interactions. Overall, these results support the potential of Fn3_5 as a structurally stable, non-antibody scaffold for selective targeting of MSLN-expressing tumors in therapeutic and diagnostic applications.

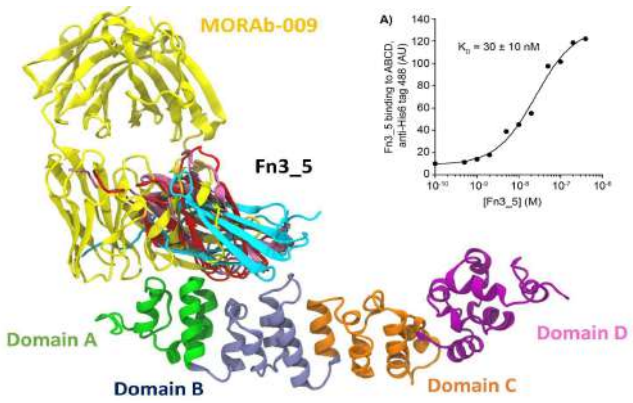

Figure: Predicted binding poses of Fn3_5 and equilibrium titration binding assay.

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Rational Design and Synthesis of hSTING Selective Agonists as Broad-Spectrum Antiviral Approach

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Keywords: hSTING, antiviral, Organic Synthesis, Drug-design.

hSTING (Stimulator of Interferon Genes) protein is a transmembrane protein that plays a pivotal role in the innate immune system. In fact, the detection of cytosolic DNA stimulates the production of cGAMP, which triggers the production of type I interferons (INF-I). [1,2] Previous studies in our laboratory have identified several monomeric benzofuran derivatives capable of activating hSTING and inhibit SARS-CoV2 viral replication. [3] With the aim to enhance selectivity and mitigate potential off-target effects, we designed indole derivatives and dimers able to occupy the target c-dinucleotide pocket, mimicking the cGAMP substrate. We hypothesize that the resulting dimeric structures will exhibit improved binding affinity and specificity for hSTING. We subjected the designed compounds to molecular docking studies to prioritize the synthesis. This dual strategy of exploring both dimeric and functionalized monomeric compounds aims to provide a comprehensive understanding of structure-activity relationships and optimize STING modulation. The synthesized compounds were submitted to biological evaluation including hSTING mediate INF-I production and the inhibition of viral replication.

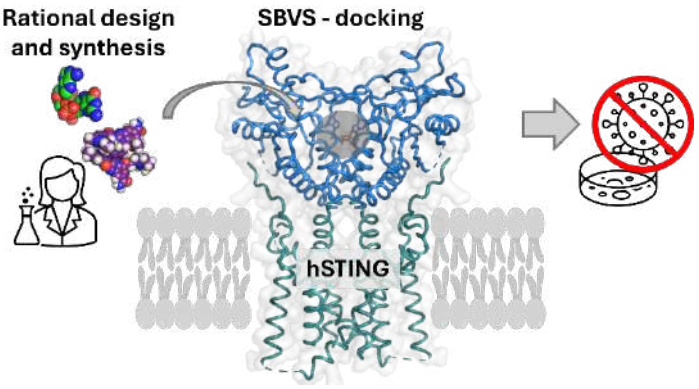

Figure 1: Project workflow

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From Repositioning to Drug Development: Design and Synthesis of Novel Captopril-Inspired MBL Inhibitors

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Keywords: Metallo beta-lactamases, antimicrobial resistance, broad-spectrum MBL inhibitors, multicomponent reactions, flow chemistry, sustainable synthesis.

The spread of antimicrobial resistance (AMR), weakening the efficacy of most of the antibiotics, represents nowadays a major global health challenge. [1] The production of β-lactamases, particularly metallo-β-lactamases (MBLs), significantly compromises the activity of β-lactam based antibiotics. [2] While substantial progress has been made in developing serine-β-lactamase inhibitors (SBLi), no metallo-β-lactamase inhibitors (MBLi) are currently available in clinical practice. [3] By applying a repositioning approach, we synthesized novel potential MBLi inspired by the captopril (**1**) structure, a widely used antihypertensive drug behaving as Angiotensin Converting Enzyme (ACE)-inhibitor. Captopril also recently demonstrated low-micromolar inhibitory activity against NDM-1, [4] one of the most clinically relevant MBL isoforms worldwide. [1] Rational design starting from captopril has been aimed at: i) retaining the thiol group, crucial for the inhibitory activity, ii) enhancing interactions within the catalytic site of the MBLs and iii) minimizing interactions with the ACE enzyme, to increase MBL inhibitory activity while reducing undesired off-target effects. Biological evaluation towards the three most relevant clinical isoforms (NDM-1, VIM-2, and IMP-1) highlighted compound **2**, exhibiting a broad-spectrum inhibitory activity in the low micromolar range, as the starting point for further scaffold elaboration. As a result, two new subseries of captopril-inspired MBLi have been designed and synthetized, aiming at exploring the chemical space to increase potency and broaden the spectrum of the activity. Different alkyl, aryl, carbamates and ureas groups were explored on the NH of the piperidyl moiety (Subseries 1, Figure 1). In parallel, the introduction of different ERG and EWG groups was explored on the phenyl ring (Subseries 2, Figure 1). Moreover, we also interrogated the outcome of an unsubstituted position 2 at the indoline ring, synthetizing compound **3** (Figure 1), devoid of the amide functionality. Compounds from subseries 2 displayed promising results with i) % of inhibition in the low micromolar range towards the three MBL isoforms tested (NDM-1, VIM-2, and IMP-1) and ii) synergistic activity with meropenem towards a panel of hyperpermeable strains of Gram-negative bacteria. Compound **3** surprisingly showed the best results among the newly conceived compounds, displaying K_i values in the nanomolar range on the three MBL isoforms. In depth biological assessment of the previously synthesized derivatives is currently ongoing. Starting from these results, future research objectives will involve the synthesis of novel compounds devoid of the amide group at the 2-position, as well as the incorporation of siderophores to improve bacterial cell permeability. [5]

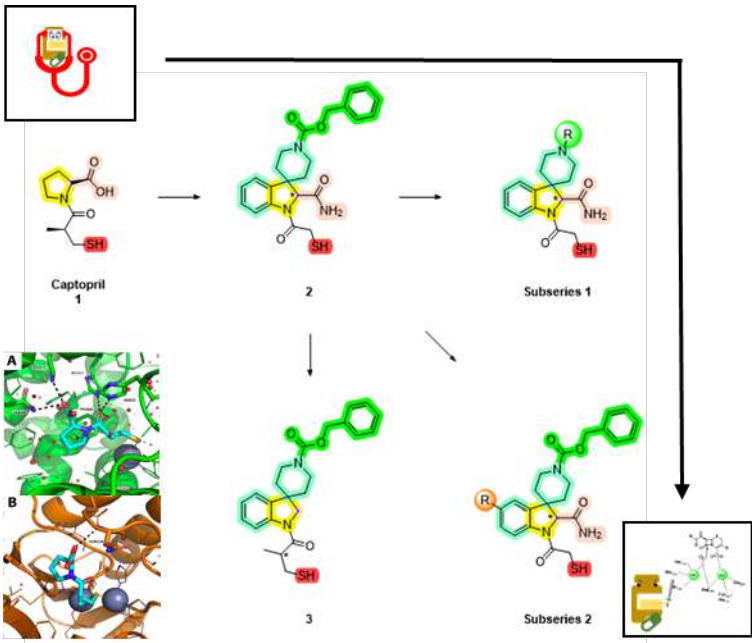

Figure 1: Summary chart of the scaffold elaboration leading from captopril (**1**) to novel promising MBL inhibitors. Comparison of the crystallographic binding mode of L-captopril in ACE (A) and L-captopril in complex with NDM-1 (B).

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Aromatic abietane diterpenoids as a promising scaffold for the development of Arnt-mediated colistin resistance inhibitors

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Keywords: antimicrobial resistance, Gram-negative bacterial infections, colistin adjuvants, natural compounds, diterpens.

Colistin is a last-resort antibiotic for treating multidrug resistant Gram-negative bacterial infections. The covalent addition of L-aminoarabinose to the lipid A moiety of LPS is the main colistin resistance mechanism in the human pathogen *Pseudomonas aeruginosa*. Since the enzyme glycosyltransferase ArnT catalyzes the last committed step of lipid A aminoarabinosylation, its inhibition represents a promising approach in facing colistin resistance. [1] Recently, a natural 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 pathogens. [2] The low compound concentration in plant material, combined with the absence of chromophore groups, makes it challenging to conduct a comprehensive microbiological characterization of this compound. Here, we considered rational procedures, following a function-oriented synthesis (FOS) approach, that enable the simplification of the ent-beyerene complex structure into drug-like synthetic molecules, thereby minimizing the structures of the pharmacophores that prove crucial for biological activity. [3] Therefore, other naturally related diterpene scaffolds were evaluated as a source of starting material to prepare a novel generation of ArnT inhibitors. In particular, this study investigated the aromatic abietane scaffold of podocarpic acid, which lacks ring D compared to the ent-beyerene one, maintains the same configuration at C-4 with respect to FDO, and features an aromatic ring C and a hydroxyl group at the C-12 position. Given its easy accessibility, podocarpic acid was selected as the starting material for synthesizing a focused library of semisynthetic oxygen and nitrogen derivatives. Combining the aromatic abietane scaffold with the functional groups essential for efficient inhibition of the ArnT activity, based on SAR studies of the *ent*-beyerene derivatives, twenty-three derivatives of podocarpic acid were synthesized to investigate the role of (i) the oxalyl group at C-16, an essential requirement for the ArnT activity inhibition of the hit compound FDO; (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; and (v) the role of the phenolic group at C-12. Several diterpene derivatives of podocarpic acid showed a significant colistin adjuvant activity, emerging as strong candidates for further experimental investigation. The aromatic abietane skeleton was identified for the first time as a privileged scaffold for the further cost-effective development of valuable colistin resistance inhibitors.

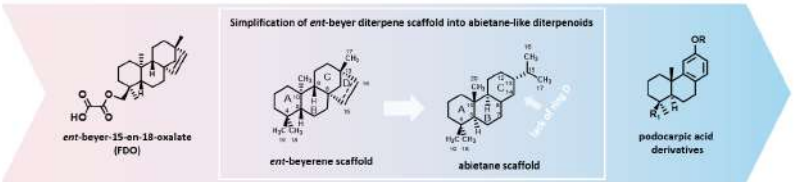

Figure: Schematic representation of the rational procedure applied for the simplification of the *ent*-beyer diterpene scaffold into abietane-like diterpenoids.

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Seeding Primary Fibroblasts and Microvascular Fragments in PLLA Scaffolds for a Regenerative Medicine Approach

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Keywords: regenerative medicine, scaffolds, chronic wounds, fibroblasts, microvascular fragments.

Chronic wounds, or ulcers, represent a significant clinical challenge due to their poor response to conventional treatments. This work proposes a regenerative medicine strategy based on PLLA (poly-L-lactic acid) scaffolds seeded with dermal fibroblasts (DFs) and adipose-derived microvascular fragments (ad-MVFs) to support tissue repair. Primary DFs were isolated from Wistar rat dermis, characterized via immunostaining, and seeded on type I collagen-coated PLLA scaffolds. Cell proliferation and morphology were evaluated through viability assays and scanning electron microscopy (SEM), confirming scaffold colonization. ad-MVFs were isolated from adipose tissue and seeded onto the scaffolds using different approaches to identify the most effective method for integration. Experiments were also performed with human DFs to assess translational potential. Ongoing studies focus on extracellular matrix production on scaffolds and co-culture experiments of DFs with ad-MVFs, aiming to combine matrix synthesis and vascularization for improved healing. This bioengineered construct is intended for in vivo testing as an innovative therapeutic option for chronic wounds.

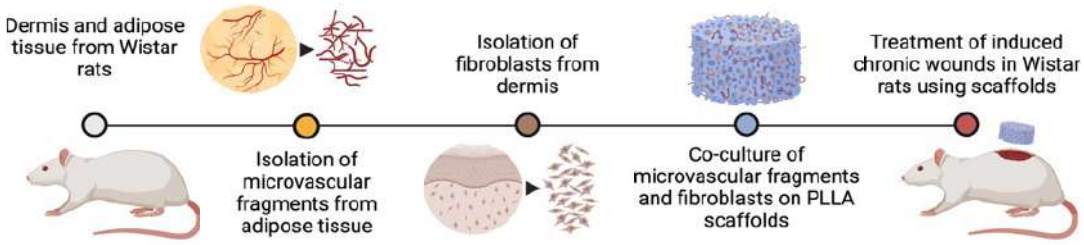

Figure: Project workflow

Computationally driven design of novel hSTING agonists for enhanced antiviral response

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Keywords: stimulator of interferon genes, antiviral effect, agonists

Viral infections trigger several mechanisms leading to the production of type I interferon and pro-inflammatory cytokines. Successively, type I IFNs activate the JAK-STAT signal transduction pathway, promoting the transcription of antiviral genes and innate immune response. [1] The human stimulator of interferon genes (STING) plays a crucial role in the antiviral response by mediating type I IFN production. hSTING is a membrane protein associated with mitochondria and endoplasmic reticulum. Since its discovery, a number of agonists have been designed, synthesised and tested to enhance its activity. [2] In this regard, our group previously published a study on hSTING agonists with antiviral activity. [3] Hence, we decided to optimize the compounds. This project focuses on the rational design of novel hSTING agonists through structure-based computational approaches. The computational workflow applied consists of 3 main steps: protein preparation, molecular docking, considering a validate protocol, and selection of the best compounds for synthesis and biological evaluation. Additionally, advanced models and algorithms have also been used to predict absorption, distribution, metabolism and excretion (ADME) properties.

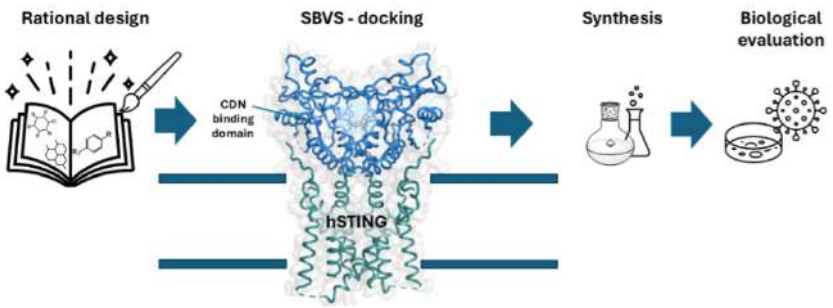

Figure 1: Project workflow.

Acknowledgments

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Spray-dried soy protein-based microparticles encapsulating Phycocyanin from *Spirulina* for inflammatory bowel disease treatment: In vivo efficacy evaluation.

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Keywords: Phycocyanin, inflammatory bowel diseases, spray drying, soybean protein, gastro-resistant microparticles.

Inflammatory bowel diseases (IBD) involve inflammation in the digestive system due to an autoimmune response. Conventional treatments often have limited effectiveness and adverse effects, prompting the search for natural compounds as alternative therapies. [1] Phycocyanin (PC), a water-soluble protein found in algae, has gained attention for its antioxidant and anti-inflammatory properties. However, PC stability is strongly affected by pH and temperature. [2] To address these challenges, this study aimed to develop and characterize soy protein-based microparticles (MPs S-PC) as an innovative delivery system for PC, employing the spray-drying technique to encapsulate and protect the molecule. Comprehensive physicochemical characterization using scanning electron microscopy, FT-IR, X-ray powder diffraction, and differential scanning calorimetry analyses confirmed the successful encapsulation of PC in an amorphous state, promoting enhanced solubility and greater chemical stability. Antioxidant activity was evaluated through in vitro radical scavenging assays, demonstrating that MPs S-PC preserved the intrinsic antioxidant potential of PC, thanks to the protective matrix formed by soy protein. Dissolution studies and ex vivo permeation experiments using porcine intestinal membranes revealed a controlled release profile of PC from the microparticles, with significant resistance to degradation in acidic gastric conditions and improved permeation in the intestinal environment. Biocompatibility tests performed on normal human dermal fibroblasts showed no cytotoxic effects, confirming the safety of the formulation. Notably, cytotoxicity assays in Caco-2 cells suggested that both free PC and MPs S-PC exerted selective antiproliferative effects, pointing to a potential dual action in managing intestinal inflammation and inhibiting malignant cell growth. The therapeutic efficacy of MPs S-PC was further confirmed in vivo using a DNBS-induced colitis rat model. Animals treated with microparticles showed a significant reduction in disease activity index, macroscopic colon damage, myeloperoxidase activity, and interleukin-6 expression compared to those treated with free PC, indicating stronger anti-inflammatory effects (Figure 1). Moreover, pharmacokinetic and bioavailability studies highlighted that the encapsulation markedly improved PC absorption, reinforcing the role of soy protein microparticles in enhancing oral delivery. In conclusion, the developed MPs S-PC system effectively overcomes the major drawbacks of phycocyanin, offering a stable, biocompatible, and efficacious oral formulation with promising applications in the treatment of inflammatory bowel disease and other chronic inflammatory pathologies.

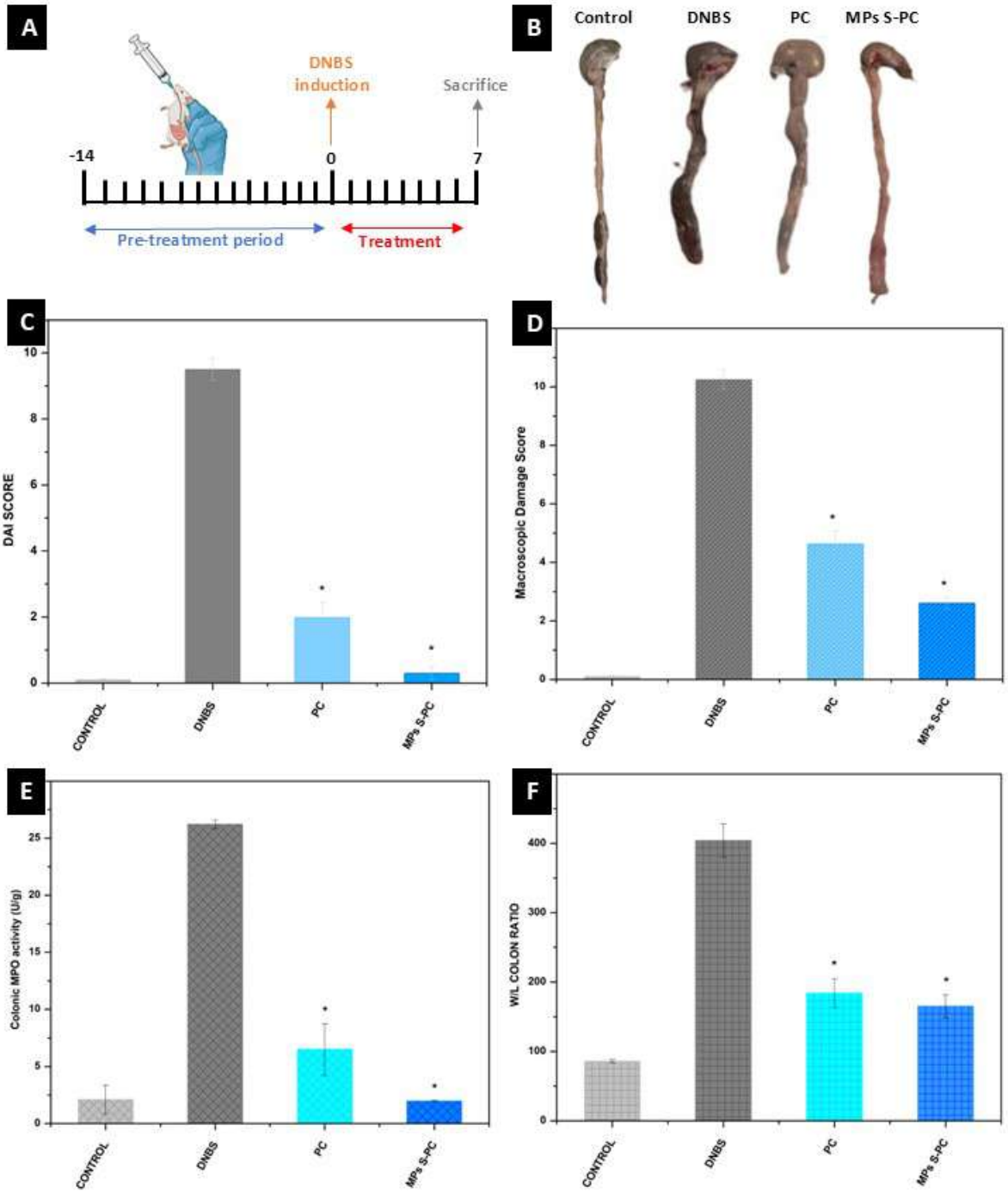

Figure 1: (A) Experimental treatment timeline. (B) Representative colons from Control, DNBS, PC, and MPs S-PC groups. (C–F) Effects of treatments on: (C) Disease activity index (DAI), (D) macroscopic damage score, (E) colonic myeloperoxidase (MPO) activity, and (F) colon weight/length ratio. *p < 0.05 vs. DNBS group.

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PDA and DHN melanin: an eco-friendly material for removing emerging contaminants from water

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Keywords: melanin, emerging contaminants, fluoroquinolones, hormones, adsorption

Interest in persistent water contaminants, including heavy metals and emerging contaminants (ECs), [1] has grown due to their environmental and health risks. ECs, present in nanogram to microgram concentrations per liter, originate from anthropogenic activities and persist in waters. Wastewater treatment plants (WWTPs) do not fully eliminate these contaminants, leading to potential toxicity concerns. Among ECs, antibiotics, particularly fluoroquinolones, [2] contribute to the spread of antimicrobial resistance in the environment, making their removal a pressing issue.[3] Hormonal pollutants from pharmaceuticals, personal care products, livestock, [4] and doping practices [5] are also abundant in water, posing ecological and health threats through bioaccumulation and biomagnification. Adsorption is an effective and cost-efficient method for removing trace organic pollutants from wastewater, offering advantages such as stability, affordability, and high performance. [6] In this context, melanins have emerged as promising bioremediation tools. These polymers natural brown or reddish pigments are synthesized via oxidation and polymerization of phenolic compounds and are widespread in different organisms: mammals, plants, fungi, and bacteria. Their ability to bind metal ions [7] and organic contaminants [8] is well documented but their use in bioremediation is still developing. [9,10] This study investigated two synthetic melanin polymers for water purification: i) DHN-melanin, derived from oxidative polymerization 1,8-dihydroxynaphthalene (DHN) and modeled after fungal melanins, and ii) PDA-melanin, synthesized from dopamine and modeled after eumelanin. [9,10] Characterization of these materials was conducted using FT-IR, XRPD, SEM, and TEM analyses.
The study assessed the efficiency of these synthetic melanins in removing ECs from tap water using HPLC-MS/MS analysis (Figure 1). [11,12] The target contaminants included five fluoroquinolone antibiotics (ofloxacin, ciprofloxacin, danofloxacin, enrofloxacin, and marbofloxacin) and five steroid hormones (estrone, 17-β-estradiol, 17-α-ethinylestradiol, progesterone, and testosterone). This work demonstrated that DHN-melanin exhibited a higher saturation capacity for fluoroquinolones than PDA-melanin, while both melanins displayed notable and instantaneous affinity for steroid hormones. Kinetic studies showed rapid adsorption, however a clear discrimination between the first and the second order kinetic models is not possible. Findings suggest that DHN-melanin and PDA-melanin are promising eco-friendly materials for the removal of ECs from water. By demonstrating their adsorption capabilities, this study contributes to the advancement of sustainable water treatment technologies to mitigate persistent environmental contamination.

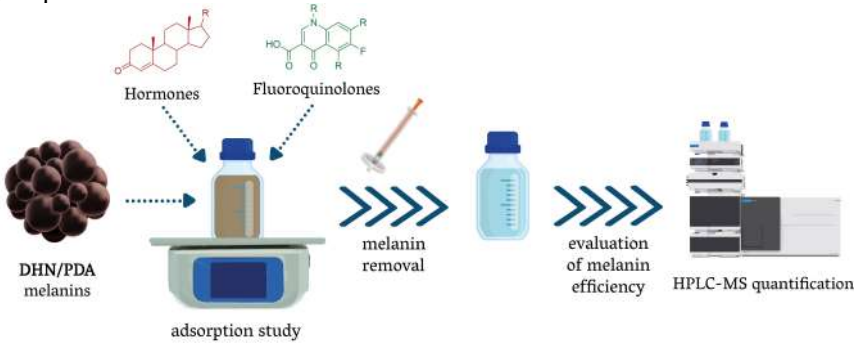

Figure 1: Experimental set up to assess the efficiency of DHN and PDA-melanins in removing hormones and fluoroquinolones from aqueous solutions using HPLC-MS/MS analysis.

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Encapsulation of Saffron in Liposomes: Formulation, Stability, and Applications

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Keywords: saffron, liposomes, cholesterol, bile salts.

Saffron (*Crocus sativus L.*) is a highly valued spice, rich in bioactive compounds such as crocins, safranal, and picrocrocin, known for their antioxidant, neuroprotective, and anti-inflammatory properties, [1-2]. However, its chemical instability and low bioavailability limit its application in pharmaceutical and nutraceutical fields. [3] Liposomal encapsulation represents an effective strategy to overcome these limitations, improving both the stability and controlled release of saffron's active compounds. [4-5] In this study, both saffron and tepals extracts were investigated to test the utility of the whole flower, thus assessing and eventually exploiting also the potential of the waste product generated in the production of the saffron spice. Saffron or tepals extracts were included in liposomes composed of saturated or unsaturated natural phospholipids in the presence or in the absence of cholesterol and bile salts (Figure 1) to evaluate their impact on the entrapment efficiency of the aggregates. The formulations were characterized in terms of vesicle size, encapsulation efficiency, stability over time, and release profile. Liposomal encapsulation improves the protection and bioavailability of saffron's bioactive compounds, making these aggregates a promising platform for pharmaceutical, cosmetic, and nutraceutical applications of the investigated extracts.

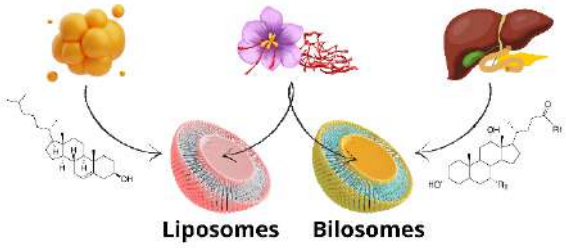

Figure 1: Structured Overview of the Research Project.

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Amination of cellulose supports for preparation of antimicrobial materials

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Keywords: Cellulose, agri-food wastes, alkoxysilane, antimicrobial composites

Cellulose makes a good alternative to petroleum-based materials because it is the most abundant natural material available in nature, with interesting properties. It is a non-toxic, biodegradable, biocompatible, and renewable organic material. For these properties, cellulose is often combined with metal nanoparticles for the development of nanotechnology, for various applications. For example, it can be used in the biomedical field to research new antimicrobial agents, which represents a global challenge due to microbial resistance to antibiotics (AMR). Cellulose does not have intrinsic antibacterial properties, but the introduction of amino pendants can extend its applications, mimicking the intrinsic bactericidal activity of chitosan. Our studies concern the development of cellulosic material functionalized with amino groups on the surface, to create carriers for ZnO nanowires (ZnO-NWs), a non-drug antimicrobial strategy. ZnO-NWs diffusing from the cellulose support in the presence of an external stimulus (like light) to facilitate their motion and interaction with bacteria. [1] In the experimental work, we functionalize cellulose in different forms, such as cellulose paper and powdered material. For the latter, we selected both a microcrystalline cellulose and a cellulose fiber derived from agri-food wastes. The functionalization was realized through the alkoxysilane technique, with different compounds, such as (3-Aminopropyl)triethoxysilane(APTES),(3-Aminopropyl)trimethoxysilane(APMS),N-(2-Aminoethyl)-3-(trimethoxysilyl)propylamine (DAMS), and 3-[2-(2-Aminoethylamino) ethylamino]propyltrimethoxysilane (TAMS), without the use of organic solvents, but only in water (Figure 1). Functionalization of cellulose via alkoxysilanes is one of the most efficient, environmentally friendly, and safe ways to introduce a wide variety of functional groups. [2] Different reaction conditions and heating sources were tested, obtaining a good grafting percentage. The obtained materials were characterized by SEM-EDX, FTIR spectroscopy. Finally, we will present preliminary results on their antimicrobial effects against both Gram-positive and Gram-negative bacteria strains.

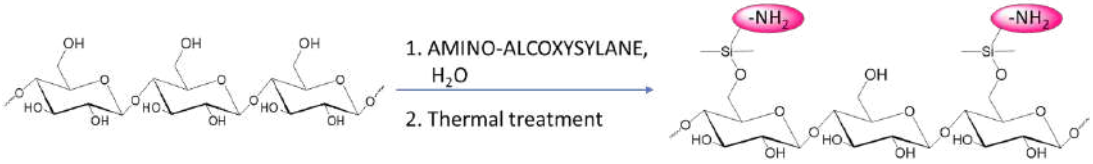

Figure 1: Functionalization with amino groups of cellulose materials

Acknowledgments

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Sustainable Biorefining of Rice waste: Extraction and Utilization of Value-added Components for Biomedical Applications

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Keywords: Biorefining, sustainable materials, Rice waste, lignin, biofilms

The efficient utilization of Rice waste is critical for addressing environmental challenges worldwide, such as waste disposal. In Italy the Annual production of rice waste, including rice husk (RH) and Rice bran (RB) is approximately 360,000 metric tons and 70,000 metric tons respectively. [1] However, there is a lack of comprehensive studies exploring low-cost and effective methods to process Rice waste. This study focuses on the biorefining of RH and RB from different varieties (Basmati and Non-Basmati) to efficiently extract the valuable components. The further objective is to prepare biobased materials by using these extracted components, more preferably Biofilms, Hydrogels and Composites with the focus on biomedical Applications. The biorefining process was performed involving alkaline peroxide treatment followed by acid precipitation and ethanol extraction to isolate cellulose, hemicellulose, lignin, silica, proteins, polysaccharides and fatty acids from RH and RB. [2] Characterization of the separated components mainly from RH was conducted using a various analytical technique, including X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The results revealed that the cellulose obtained was in the form of bundles of fiber-like structures with a fiber width of approximately 10µm. The cellulose demonstrated a high degree of crystallinity (60%) and it could be a valuable source for the production of advanced materials. Additionally, hemicellulose, silica and lignin were observed as small particles with non-uniform shapes, exhibiting amorphous structures. Lignin offers potential in the production of biofilms and hydrogels for wound healing applications and could be more efficient candidate against the infectious bacteria. Due to the antibacterial properties of lignin, [3] it was selected to prepare the biofilms and hydrogels for wound healing applications. Multiple sets of experiments, mixing lignin with some commercial polymers (carboxymethyl cellulose, starch and polyvinyl alcohol) were performed to attain the best formulation of biofilms. Preliminary tests like swelling ability, water solubility and mechanical properties have been done to check the behavior and further analysis are ongoing. The biorefining process utilized in this study is environmentally friendly, requiring relatively low energy input and avoiding the use of dangerous chemicals. This approach is not only cost-effective but also scalable, making it suitable for large-scale industrial applications. The successful isolation of cellulose, hemicellulose, lignin, and silica demonstrates the potential of RH as a sustainable resource of valuable materials. The separation and recovery of valuable components from RH and RB can contribute to reducing waste disposal issues in agricultural industries while providing renewable resources for a wide range of applications.

Figure 1: Extracted Components from RH

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MIP-based sensor for the determination of PFOA in wastewater samples

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Keywords: Perfluorooctanoic acid, Molecularly Imprinted Polymers, Electrochemical sensor, Water.

Per- and polyfluoroalkyl substances (PFAS) are a class of fluorinated synthetic organic compounds used in various industrial applications. They are considered emerging pollutants and are known as 'forever chemicals' due to their high persistence in the environment. These substances pose risks to both ecosystems and human health, making their detection and monitoring essential. Therefore, the development of analytical tools with short response times, low cost, and ease of use is crucial. In recent years, particular attention has been given to perfluorooctanoic acid (PFOA), which consists of an eight-carbon fluorinated chain with a terminal carboxyl group. Although still used as a surfactant in the synthesis of fluorinated compounds, its use has been significantly restricted to a maximum of 25 ppb in the production of other materials, as specified in EU Regulation 2017/1000. In this context, the aim of this work was to develop an electrochemical sensor based on molecularly imprinted polymers (MIPs) for the detection of PFOA in environmental samples, specifically in water samples from a wastewater treatment plant. Particularly, the electrode surface was modified by electropolymerizing monomers of *o*-phenylenediamine (*o*-PD) in the presence of PFOA to create active sites able to recognize the analyte. The analytical protocol was subjected to various optimizations to enhance the overall performances. Different electrode materials, including glassy carbon and gold, were tested and compared by studying their electrochemical properties, their behavior during the electropolymerization process, and their sensitivities in the detection of PFOA after the modification with the molecularly imprinted poly(*o*-PD). In addition, the response of the sensor towards interferents was tested, highlighting good selectivity. The sensor was then applied to the detection of PFOA in real matrices, working with wastewater samples from water treatment plants, demonstrating great efficacy and good sensitivities in complex environmental samples.

Acknowledgments

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DNA repair monitoring through an electrochemical scaffold sensor

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Keywords: DNA glycosylase, DNA damage, scaffold sensor.

Exposure to ionizing radiation produces radicals and a state of oxidative stress resulting in base lesions. Oxidized bases lead to abasic sites and accumulation of such damage can result in mutagenesis and cell death. Base excision repair (BER) addresses these lesions involving key DNA glycosylases enzymes such as OGG1 and MUTYH (MutM and MutY bacterial homologs respectively) which are responsible for the recognition, removal and repair of 8-oxoguanine (8-oxoG) lesion. [1] The presence of 8-oxoG in DNA causes G to T transversions, in the BER pathway. Thus, DNA glycosylase enzymes aid to prevention of mutagenesis caused by oxidative DNA damage. OGG1 removes 8-oxoG from 8-oxoG•C base pairs, and MUTYH removes A from 8-oxoG•A base pairs, both generating apurinic/apyrimidinic sites in the DNA. By excising the mismatched adenine, MUTYH accelerates the subsequent downstream repair processes in BER pathway that restores genomic integrity, thereby diminishing the risk of G:C to T:A transversion mutations and oncogenic transformations. [2] Employing programmable nucleic acid nano switches as diagnostic tool, the electrochemical (EC) bioanalytical platform is envisioned to be developed in the present study demonstrating molecular activities underlying the MUTYH repair function. MUTYH mediated recognition of 8-oxoG lesion, installed within single strand DNA labelled with a methylene blue (MB) reporter, results in sensitive electrochemical signal changes and can be conveniently detected in-vitro. The study will help to understand MUTYH's function in DNA repair for developing targeted therapies to prevent cancer, specifically MUTYH-associated polyposis, a genetic colorectal cancer syndrome and thus contributing to better management of the disease.

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Aphanizomenon flos-aquae (AFA), a new potential therapeutic microalgae against aberrant DNA methylation and DNA damage

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Keywords: Methylation, microalgae, inflammation, epigenetics

Aphanizomenon flos-aquae (AFA), a cyanobacterial species thriving in the Upper Klamath Lake located in Oregon, USA, is revered as a nutritional powerhouse and is often consumed as a "superfood". [1] AFA has garnered attention for its potential therapeutic benefits in various health conditions, but its nutrigenomic and epigenetic impacts remain underexplored. It is known that in inflamed tissues, alterations in DNA methylation patterns have been observed, often characterized by global hypomethylation and site-specific hypermethylation. [2] Understanding the intricate interplay between DNA methylation and inflammation is crucial to identify potential targets for therapeutic intervention since modulating inflammation through epigenetic processes may be a promising strategy to reduce basal inflammation and prevent various pathologies. [3] Furthermore, it is known that natural compounds show a reversal power of DNA demethylation/damage, caused e.g. by the natural pollutant Arsenic. [4] GammaH2AX is a phosphorylated form of the histone protein H2AX, which plays a crucial role in the cellular response to DNA damage. When DNA double-strand breaks occur, the H2AX protein is rapidly phosphorylated at a specific site, leading to the formation of gammaH2AX. This modification serves as a signal for the recruitment of DNA repair proteins to the site of damage, facilitating the repair process and maintaining genomic stability. Essentially, gammaH2AX acts as a marker for DNA damage and is important for the cellular response to stress and repair mechanisms. Our study investigates the potential epigenetic mechanisms of AFA, focusing on its ability to modulate DNA methylation, a key regulatory process in gene expression and to influence the DNA-repair mechanism. Specifically, we examined the influence of AFA on the DNA methylation status of genes encoding pro-inflammatory interleukins, as these cytokines play a crucial role in immune response modulation and inflammation. Furthermore, consequently treatment with Arsenic in concentration similar to that can be assumed by drinking water, we evaluated if AFA is able to modulate Arsenic-induced DNA damage. Given the known impact of AFA on inflammatory markers, we aimed to determine whether the effects of AFA involve direct or indirect modulation of DNA methylation patterns in genes associated with inflammation. Our findings, presented here for the first time, reveal the capacity of AFA to influence DNA methylation, with implications for its role in cellular regulatory processes and to protect from DNA damage inevitably induced by a chemical insult such as Arsenic. These results warrant further investigation into precise mechanisms of action of AFA and its potential in clinical applications targeting inflammation-related pathways and DNA reparation mechanism.

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Optimizing Hybridization Temperature in Nucleic Acid-Based Point-of-Care Diagnostics

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Keywords: PoCT, NA, hybridization temperature, salt influence

Point-of-Care Testing (PoCT) enables rapid diagnostics with minimal infrastructure, providing high specificity and multiplexing capabilities essential for advancing healthcare delivery. [1,2] Nucleic Acid (NA) – based PoCT rely on several critical processes, including sample preparation, probe immobilization, hybridization, and signal detection. Among these, hybridization represents the cornerstone step where specific target sequences bind to complementary probes to generate test results. Hybridization efficiency is influenced by the hybridization temperature (T_H) – the temperature at which NA probes and targets hybridize most efficiently – which directly impacts diagnostic accuracy. Precise optimization of T_H is crucial: temperatures above the optimal range may lead to weak or incomplete hybridization, while temperatures below it may cause mispriming, self-folding, homo-binding, and heteroduplex formation. The optimal T_H depends on multiple factors, including nucleotide sequence length and composition (particularly GC content), probe concentration, and ionic strength. [3,4] In this study, we aimed to evaluate the impact of salt concentration on T_H by monitoring SYBR Green I intercalation into the double-stranded NAs, producing a detectable fluorescence signal. T_H was defined as the temperature at which 50 % of the maximum fluorescence was observed. Complementary 21-nucleotide single-stranded NAs sequences (10 µM) were tested across NaCl concentrations ranging from 1 µM to 1 M using a temperature gradient (80 °C to 55 °C in 0.5 °C decrements). As shown in Figure 1, T_H exhibited a positive correlation with salt concentration from 1 µM to 10 mM NaCl, increasing from 55 °C to 71 °C. The plateau observed between 10 mM and 100 mM NaCl suggests a saturation point, beyond which further increases in salt concentration have minimal impact on T_H. At low salt concentrations, electrostatic repulsion between the negatively charged phosphate backbones reduces hybridization stability, requiring lower annealing temperatures. As salt concentration increases, charge shielding minimizes this repulsion, promoting more stable hybridization at higher temperatures. However, the observed decrease in T_H at extremely high salt concentration (1 M) suggests the existence of an optimal salt range, beyond which excessive charge shielding may negatively affect hybridization efficiency. These findings offer key insights for optimizing salt concentrations in NA-based PoCT, improving diagnostic accuracy and reliability to better align with the REASSURED criteria and advance global healthcare initiatives. [5]

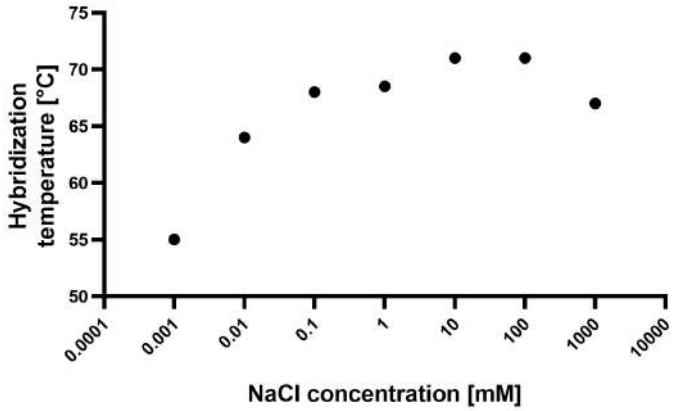

Figure 1: Dependence of T_H on the salt concentration, with double-stranded nucleic acids solubilized in NaCl at concentrations ranging from 1 µM to 1 M.

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DNA hypomethylation compromises chromosomal stability and drives defects in cell nucleus structure and mechanics

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Keywords: DNA methylation, heterochromatin, nuclear architecture, genomic instability, cell transformation.

DNA methylation is an epigenetic modification that facilitates chromatin compaction, regulating both chromatin structure and gene expression. Global loss of DNA methylation occurring at gene-poor repetitive sequences - the most abundant elements of the genome - is a hallmark of genomic instability frequently observed in various types of cancer. [1,2] Beyond its role in transcriptional regulation, DNA methylation is essential in maintaining repetitive elements in a heterochromatic, compacted state. [2] In this context, heterochromatin has a defined spatial organization within the nucleus, predominantly localizing at the nuclear periphery, where - together with the nuclear lamina - it regulates nuclear morphology and mechanical properties, ensuring nuclear stiffness. [3] Consequently, a widespread DNA hypomethylation, by affecting heterochromatin, could impact both nuclear architecture and mechanics (Fig. 1). [4] This study explores the relationship between DNA methylation and nuclear integrity, proposing a novel mechanism by which DNA hypomethylation contributes to genomic instability and tumorigenesis. To investigate this, we used the non-tumor immortalized RPE-1 cell line, engineered to induce DNA hypomethylation by the rapid degradation of the maintenance DNA methyltransferase DNMT1 for several cell cycles. [5] Our findings show that DNA hypomethylation leads to aneuploidy and accumulation of misaligned chromosomes during metaphase, indicating that proper DNA methylation is crucial in supporting chromosome segregation. Furthermore, microscopy observations reveal striking nuclear morphological changes upon DNA methylation loss, as evidenced by increased nuclear size and a higher incidence of blebbing. Western blotting and immunofluorescence analyses of the nuclear envelope components LaminB1 and LBR demonstrate a reduction in protein levels and impaired localization, suggesting that the observed nuclear phenotypic alterations might be a consequence of the rearrangement in nuclear envelope composition. Lastly, biophysical approaches further reveal that DNA hypomethylation weakens nuclear envelope structural integrity. FLIM-based measurements using a fluorescent membrane mechanosensor reveal a reduced nuclear membrane tension in hypomethylated nuclei. Additionally, nucleus indentation experiments with optical tweezers show a decrease in nuclear stiffness. Altogether, our results demonstrate that DNA hypomethylation impairs chromosome dynamics during mitosis and compromises nuclear envelope integrity, probably resulting in the observed structural and mechanical defects that enhance nuclear deformability and may facilitate cell transformation. Overall, this study sheds light on the role of DNA methylation in safeguarding genome stability by preserving nuclear architecture and mechanical properties, proposing that DNA hypomethylation could act as an oncogenic event.

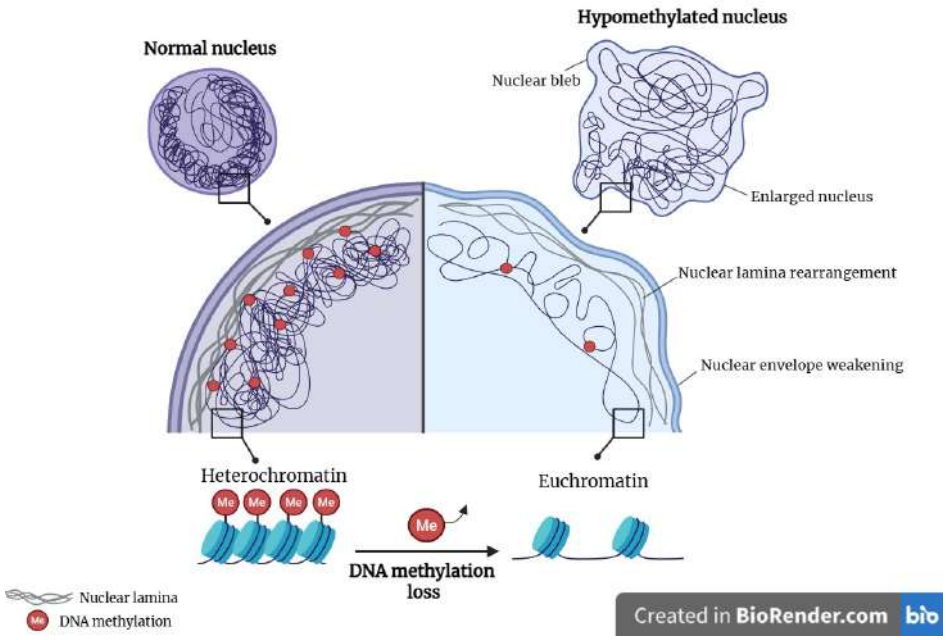

Figure 1: Schematic representation of DNA hypomethylation effects on cell nucleus.

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DNA methylation loss leads to failure in chromosome segregation faithfulness

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Keywords: DNA methylation, Cancer, Chromosome segregation, aneuploidy, Centromere

DNMT1 is the enzyme involved in the maintenance of DNA methylation. Autosomal dominant cerebellar ataxia, deafness and dementia have been characterised by DNMT1 mutation, impairing DNA binding and thus DNA methylation maintenance. DNA methylation is an epigenetic modification that occurs on the DNA. Its loss correlates with autoimmune diseases such as lupus and neuropsychiatric disorders. [1] DNA methylation is known to regulate gene expression. However, DNA methylation is also present at DNA Repetitive elements which are gene poor. DNA hypomethylation of repetitive DNA has been correlated with aneuploidy and it is speculated to induce cancer progression. [2] While normal cells, such as RPE-1 cells arrest the cell cycle when the prolonged absence of DNMT1 triggers a toxic level of DNA methylation loss, [3] tumour cells bypass this checkpoint. This suggests that DNA hypomethylation could contribute to the genomic instability typical of cancer cells. Interestingly, DNA methylation normally characterizes centromere repetitive DNA but the connection with the aneuploidy is still unclear. Faithful chromosome segregation relies on the attachment of the microtubules of the mitotic spindle to the kinetochore which, in turn, is supported and recruited by the centromere. Thus, any alteration of the repetitive DNA at centromere may affect the attachments and the fidelity of chromosome segregation. To better understand DNA methylation role in this event, we used the Auxin Inducible Degron (AID) strategy to induce the degradation of DNMT1 in DLD-1 tumoral cells and achieve DNA hypomethylation. [4] Since genome stability is based on faithful chromosome segregation, we focused on the proteins involved in microtubule-kinetochore attachment. Upon DNA hypomethylation, we observed aneuploidy induction and loss of chromatid cohesion at the centromere. Also, we observed an increase of centromere and kinetochore proteins. Similarly, AuroraB kinase, involved in error correction, was increased at kinetochore in absence of attachments. Astrin, which instead senses the attachment, [5] was increased as well at kinetochore. Moreover, cold treatment assay did not show loss of attachment stability. All these suggest that despite its increase, AuroraB kinase is not able to rescue uncongressed and misaligned chromosomes. Our study shows that DNA methylation preserves the ploidy of cells by regulating chromosome segregation. Further investigation is needed to clarify the mechanism; however, our current results unveil the importance of DNA methylation in chromosome stability.

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POSTER

Targeting Nonsense Mutations: NV848, NV914, and NV930 for REP1 protein restoration in Choroideremia

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Keywords: genetic disease, nonsense mutation, suppression therapy, oxadiazole, readthrough.

Choroideremia is a rare, X-linked genetic disease caused by mutations in the CHM gene on the X chromosome, with an estimated incidence of approximately 1:50,000 to 1:100,000 worldwide. This disease is characterized by progressive night blindness and degeneration of the visual fields, initially affecting the peripheral areas and later the central ones. The progressive degeneration of the retinal pigment epithelium (RPE), photoreceptors, and choroid results in significant peripheral field loss in adolescence and adulthood, central visual loss during adulthood, and total blindness in the last decade of life. The CHM gene encodes the Rab Escort Protein 1, also known as REP1, which is the A subunit of the Rab Geranyl-geranyl transferase II (RabGGTII), involved in the geranylation of Rab proteins. [1] Among the several mutations, 34% are represented by nonsense mutations in the CHM gene; these lead to the formation of a premature termination codon (PTC) in the CHM mRNA coding sequence, with the absence or a truncated and non-functional REP1 protein. REP1 protein is not only involved in the vesicular trafficking and the correct prenylation on Rab protein but also in several pathways involved in cancer, oxidative stress, autophagy, and apoptosis. [1,2,3] Currently, there is no cure or treatment for nonsense mutations; however, promising developments are underway. Still, promising results come from suppression therapy, based on the use of translational readthrough inducing drugs (TRIDs), that can overall the PTC when the ribosome go on it, introducing a near-cognate tRNA in correspondence of the PTC, allowing the continuation of the translation instead of termination. Recently, three TRID molecules (NV848, NV914, and NV930), derivatives of Ataluren, a well-known readthrough agent, have shown fascinating results in vitro and vivo. [4,5,6] Our work focuses on studying the possible readthrough activity and the effect of these molecules on different Choroideremia nonsense models. To assess the rescue of the REP1 protein expression, immunofluorescence analyses were performed after 48 hours of treatment. Furthermore, to evaluate their activity in primary choroideremia cell cultures, fibroblasts from CHM^{R253X} patients were subjected to a 72-hour chronic treatment, followed by immunofluorescence evaluation of REP1 protein rescue. The results demonstrate the successful rescue of the REP1 protein in all analyzed models, confirming the promising potential of these compounds in counteracting nonsense mutations.

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Peptides functionalized metal nanoparticles for dynamic cell interaction

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Keywords: Laser ablation**,** nanoparticles, nanomedicine, cell interaction, gold-iron

The aim of this project is the development of a dynamic vector at the cellular level, utilizing gold-based metal nanoparticles (NPs) functionalized with specifically designed peptides. [1,2] These peptides not only improve biocompatibility but also enable the NPs to respond to external stimuli, such as pH variations.
The synthesis of these NPs is achieved through laser ablation in liquid of a metallic target [3] followed by a secondary laser-induced fragmentation process. During this last stage, the colloidal suspension is irradiated with UV nanosecond laser pulses, inducing photofragmentation and enabling precise control over NPs size and size distribution. This method ensures a high degree of purity and avoids chemical contaminants typically associated with conventional wet-chemical synthesis techniques.
Following the synthesis, the NPs are functionalized with peptides obtained via solid-phase peptide synthesis (SPPS). The selected peptide sequences are customized to respond to pH variations due to the presence of multiple basic residues (e.g., lysine), while a thioester group facilitates the formation of a covalent bond between thiol(-SH) groups and gold atoms. The presence of these peptides contributes not only to the stabilization of the colloidal solution but also enables reversible aggregation at physiological pH and disaggregation under acidic conditions, which are typical of the extracellular environment of cancer cells. This behavior enhances intracellular localization, promoting the migration of NPs near the nucleus and increasing their potential efficacy for therapeutic applications.
The physicochemical characterization of the NPs, alongside systematic evaluation of their stability, surface properties, and functional performance are performed. Subsequently, in vitro studies will assess their biocompatibility, cytotoxicity, and potential as contrast agents for multimodal imaging. Furthermore, their theranostic capabilities will be investigated in the context of radiosensitization for X-ray radiotherapy, aiming to improve therapeutic outcomes in oncological treatments. This study presents an innovative approach to nanoparticle design, integrating advanced nanofabrication techniques with targeted biofunctionalization strategies to enhance precision medicine applications.[4]

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Does Environmental Warming Enhance the Toxic Effects of Phthalates on Marine Life?

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Keywords: climate change; marine pollution; phthalic acid esters; plastic additives; sea urchin embryos

Marine heatwaves driven by climate change pose a significant threat to marine organisms, exacerbating biodiversity loss in synergy with other anthropogenic stressors, such as chemical contamination. This study investigated the effects of extreme thermal events and phthalic acid esters (PAEs) pollution, both independently and in combination, on the embryonic development of the sea urchins *Arbacia lixula*, which were collected from the Capo Gallo-Isola delle Femmine Marine Protected Area near Palermo, Italy.
Additionally, it examines whether the species' optimal developmental temperature (24°C) enhances tolerance to PAEs compared to ambient conditions (18°C).
Fertilization followed a previous methodology [1], achieving a success rate > 95%. Fertilized eggs (4000 eggs/mL) were distributed in glass Petri dishes and incubated at 18°C and 24°C with PAE concentrations ranging from 0 to 50 mg/L until reaching the pluteus stage (48 h). A standard solution containing six PAEs (2000 mg/L each) was initially diluted in hexane to prepare the different exposure concentrations. The solvent was then evaporated under a gentle nitrogen flow, and the residue was reconstituted in 40 µL of dimethyl sulfoxide (DMSO), i.e., 0.4% in 10 mL of filtered seawater. DMSO was selected as a carrier due to its minimal toxicity and biocompatibility. [2] The ecotoxicological assessment revealed that heatwave conditions significantly amplified PAEs-induced toxicity, leading to a 76% decrease in median effective concentration (EC_{50}) values. Morphological analysis showed that the combined impact of elevated temperature and PAEs exposure resulted in an increase in developmental abnormalities and a reduction in skeletal growth. Biochemical analysis further indicated that temperature was the primary factor affecting the activity of stress response enzymes, including alkaline phosphatase, esterase, and peroxidase. Statistical analysis (ANOVA) confirmed that both temperature ($p = 0.0045$) and PAEs concentration ($p < 0.0001$) significantly affected development.
These findings suggest that heatwaves and PAEs contamination jointly reduce *A. lixula* embryo tolerance. Furthermore, the study suggests that 24°C may not be the optimal developmental temperature for the southwestern Mediterranean population of *A. lixula*. The results underscore the necessity of integrative ecotoxicological assessments, as evaluations based on single stressors or biological levels may lead to misleading conclusions regarding marine organism health and thermal tolerance.
With rising temperatures, endocrine-active substances may be released from polymers in which they are mixed (e.g., plastics) or biosynthesized by various organisms as secondary metabolites. [3] This could increase environmental exposure, leading to bioaccumulation in the food chain and potential human health risks. Further research is needed to assess long-term impacts.

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A novel chemometric strategy for *in-situ* electrical conductivity prediction of tap water in a flow system

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Keywords: chemometrics, PLS, impedance, conductivity, tap water

Electrical conductivity is a fundamental property to measure when water samples are collected for chemical analyses; the technological advancements in instrumentation allow to perform rapid and reliable conductivity measurements *in-situ*. In addition, many methods have been reported for calculating the conductivity of a natural water sample from its chemical composition. [1] In this study, we are presenting a multivariate strategy for conductivity determination coupling Design of Experiments (DoE) with Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS), relying on electrical impedance measurements on tap water in a flow system (NEMO, online water-quality monitoring instrument, national patent N. 102022000017490 of 08/23/2022 whose title is "DISPOSITIVO PER ANALISI CHIMICO-FISICA-BIOLOGICA DELL'ACQUA").
As for experimental planification by DoE, we considered as variables the amplitude of the sinusoidal potential (20-100 mV), the amplification of the output signal (1-40 kΩ) and the working electrode type. D-Optimal Design was used to find the best training and validation sets from a list of candidate experiments and, per each set of operating conditions, we recorded electrical impedance in a frequency range from 2 to 150 kHz, applying a potential for the direct current equal to 0 mV. The resulting spectra (Bode and Nyquist plots merged with a low-level data fusion) were submitted to PCA and PLS regression to compute two responses: the explained variance on PC1 and the Root Mean Square Error in Cross-Validation (RMSECV) from PLS models.
Instead of preparing simulated water samples, we used eight types of bottled water with different chemical compositions and conductivities, in a range between 15 and 767 μScm^{-1}. Impedance measurements were performed at 30 L/h flow speed and in different days and parts of the day to jointly evaluate reproducibility and repeatability. Some sets of operating conditions were satisfying: the PCA loading plot (Figure 1a) showed orthogonality between real and imaginary components in Nyquist data (same for magnitude and phase in Bode visualization) as showed in other studies. [2] In addition, there was also a good conductivity-dependant separation of the samples on the score plot (Figure 1b) and a satisfactory reduction of experimental variability. Lastly, the same settings resulted in well performing PLS models despite matrix effect and flow influence (Figure 1c). The use of scaling pre-processing enhances the contribution of low frequency phase components in conductivity prediction, allowing to expand the applicability range in comparison with magnitude-based univariate approaches. This new strategy renovates the way of measuring electrical conductivity for tap water, giving a robust and reliable alternative to the traditional conductivity cell; jointly with the possibility of working in an automated and remote-controlled flow system, it makes monitoring easier without the need for human intervention.

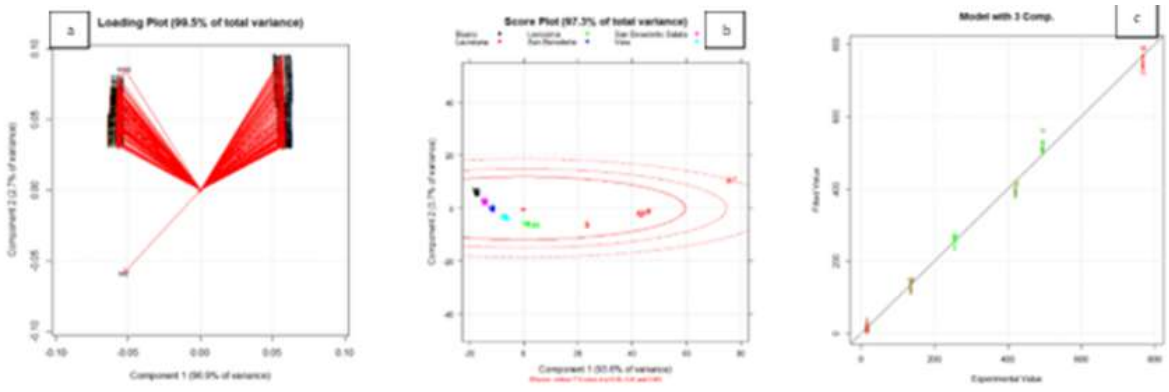

FIGURE 1: a) the loading plot for Nyquist PCA, b) the score plot for merged Nyquist-Bode PCA, c) PLS model from one of the best performing levels.

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Design of ZnO nanostructures for the Electrochemical Sensing of Creatinine in Aqueous solution

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Keywords: Sensing, Creatinine, Zinc Oxide, impedance electrochemical spectroscopy.

Creatinine (CRE) is a metabolic waste product generated in the human body, whose detection is crucial for assessing an individual's health status. By measuring the concentration of CRE in body fluids, it is possible to diagnose potential renal dysfunctions and prevent complications arising from the onset of diseases. [1] Currently, CRE is detected through well-established analytical methods, such as colorimetric techniques (e.g., Jaffe method) or enzymatic methods. However, these approaches suffer from narrow concentration range, susceptibility to degradation, and high costs. [2] In this context, non-enzymatic sensors (NES) represent a viable alternative, offering advantages such as good stability after repeated use, high selectivity, and reduced costs depending on the materials used. [3] In this study, a sensor for CRE has been designed based on its interaction with zinc-based compounds, which can be detected using electrochemical methods. Specifically, a ZnO/ITO/PET-based sensor was synthesized following a previous protocol outlined in a recent study on smart wearable devices. [4] The sensor detection was obtained using a three-electrode electrochemical cell in a working solution containing the redox couple 1 mM $[Fe(CN)_6]^{3-/4-}$,. Cyclic voltammetry was performed on this setup to assess the reversibility of the redox phenomena occurring on the WE surface, while electrochemical impedance spectroscopy (EIS) measurements were carried out with increasing concentrations of CRE (10μM < [CRE] < 100μM). The analysis of the Nyquist plots showed that the R_{cp} values, obtained through a Randles circuit, in relation to the concentration of CRE in solution, in accordance with previous results. [5] The interaction between the target molecule and the ZnO sensor surface could be attributed to the material's surface morphology, providing a large surface area for CRE adsorption and/or to the formation of Zn^{2+}-CRE complexes [5], which may in proximity to the sensor surface due to the release of Zn^{2+} ions into the solution. Future experiments could aim to better understand the nature of this interaction. It is also important to evaluate how potential physiological interferents might alter the instrumental responses, to assess the sensor's selectivity. Additionally, the piezoelectric properties of ZnO are being studied to improve the sensitivity in the detection of CRE and other analytes of similar nature and significance. This type of research will be crucial in the development and implementation of point-of-care devices.

Acknowledgements

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The effect of the temperature on the interaction between SLNPs loading a mRNA and target cells

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Keywords: Solid Lipid Nanoparticles, vaccines, cytotoxicity, A549

The development of mRNA vaccines associated with nanocarriers has been a breakthrough against Covid19 pandemic. The efficacy and safety of vaccines is everywhere proven. The use of solid lipid nanoparticles (SLNPs) to enable the delivery of the mRNA is beneficial thanks to the protection of SLNPs from the degradative action of ribonucleases in vivo, extending the blood circulation time of the vaccine by reducing their clearance. However, the bio-nano interaction of solid lipid nanoparticles with target cells still requires further investigations. The goal of this study is to evaluate chemical stability and biological ability of mRNA-based vaccines to penetrate in cells and their behavior once internalized. It is important to understand whether and how these vaccines can remain safe and effective in the face of various stressors in order to promote vaccination campaigns worldwide and ensure global protection. Due to the relevance for the global health, in this study we focused the attention on stability and efficiency of vaccines after exposure to thermal stress. DLS analysis demonstrated that Pfizer SLNPs exposed to 40° for 8 days modified important physico-chemical features. [1] Subsequently, a dose-dependent study to assess the cytotoxicity on A549 cells has been carried out with stressed and non-stressed SLNPs, performing MTT assay, at different concentrations from 2,5 µg/mL to 0,078 µg/mL. In addition, hydrogen peroxide levels were detected by means of ROS-Glo assay. Moreover, mRNA-SLNPs were labelled by using DiD fluorophore to make them detectable in microscopy studies. Then, cell membrane staining and immunostaining of the main intracellular vesicles were performed to study internalization kinetic and colocalization rate of the vaccines with intracellular vesicles. Concerning the results, toxicity studies reported that, at 3, 24 and 96 hours of incubation, Pfizer triggered major toxicity than Pfizer stress, resulting in a minor cellular vitality. As for the ROS-Glo assay, the results showed no significant production levels of hydrogen peroxide over the positive control. Finally, fluorescence microscopy analyses revealed different kinetics of internalization and colocalization with endosomes and lysosomes: Pfizer SLNPs had a gradual entry and increase overtime, while Pfizer stress SNLPs showed an initial peak of internalization and then a decrease at longer timepoints. In conclusion, mRNA-based vaccines encapsulated in SLNPs represent a promising strategy to ameliorate stability of vaccine's formulation. During this study, the stability of COVID-19 vaccines has been investigated showing differences between Pfizer and Pfizer stress in terms of toxicity and different behavior once internalized. Further studies are necessary to better understand major intracellular interactions of these mRNA-based vaccines and their contribution to molecular pathways.

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Enabling early detection of ovarian cancer using a novel electrochemical sensing platform

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Keywords: Immunosensor, Ovarian cancer, CA125, HE4, Multiplex sensing

Ovarian cancer (OC), frequently known as the "silent killer," is among the top ten cancer mortality reason affecting 300,000 women annually. [1] The present available therapies can heal up to 90% of stage1 patients. However, only 20% of Cancers are detected at this stage. Survival is highly dependent on stage of disease. By increasing the stages to 4, the treatment rate drops significantly. [2,3] Our research involves the development of a disruptive multiplexed near-patient diagnostic solution, to enable early detection of Stage I and II OC using two different biomarkers.
Gold flexile electrodes were modified with a polymer layer and the antibodies of interest, anti-HE4 and anti-CA125, were immobilized on the surface of electrodes. Preliminary results show that the sensor has a linear range of 0.1 fg/ml to 10 pg/ml and 10 fg/ml to 1 ng/ml for HE4 and CA125 respectively. The sensitivity of 0.31 and 0.53 $K\Omega.mm^2/ml$ were calculated for HE4 and CA125 in sequence.
This diagnostic device shows a novel high sensitivity electrochemical sensor platform, in which the combination of multiple biomarkers facilitates a high level of accuracy.

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Evaluation of the biocidal activity of Origanum vulgare L. essential oil in nanoemulsion

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Keywords: essential oil, nanoemulsion, antimicrobial activity

Natural compounds from plants could replace or support chemically synthetic biocides in reducing the number of pathogenic microorganisms under defined conditions. [1] In particular, Essential Oils (EOs) have attracted increased interest due to their biocompatible, rather innocuous nature and interesting antimicrobial properties. [2] In this work, the efficacy of both negatively and positively charged lipid-based nanoemulsions (NEs) of *Origanum vulgare L.* (OEO) and its main component carvacrol (CAR), was determined against C. albicans. NEs were prepared using 60 µL OEO, 20mg soy phosphatidylcholine (soy-PC), phenylacetic acid, PAA; benzyl alcohol, BA; and benzalkonium chloride, BK added to provide electric charge. [3] Carvacrol was also loaded into NEs and its effects were studied and compared with a dispersion of unformulated OEO in an aqueous Tween 80 solution. GC/MS analysis determined the chemical composition of the NEs. The formulations were tested for their evaporation ability to verify if the solvent displacement technique provide a feasible stability technology for the OEO. In particular, the chemical profile of OEO by HS-GC/MS analysis was established. The obtained results were compared with the head space composition of the non-formulated bulk oil. Antimicrobial activity was evaluated against three vaginal isolates of *C. albicans*, using the broth microdilution for MIC and MFC. Considering that the minimum amount of soy-PC calculated to form a monolayer around 200 nm oil droplets is 13 mg, four different NEs were prepared by varying the amount of soy-PC from 5 to 20 mg. The results of DLS characterization show that the droplet size, PDI and zeta potential values of the NE_OEOSoyPC were found to be 220 nm, 0.119 and –28.4 mV, and they remained stable over time. Investigating soy PC with a cationic-charged co-surfactant, (BK) and emulsifiers (PAA). Combining BA with soy PC produces similar results. All nanoemulsions remained stable during storage and had good droplet growth stability. The optimized NEs were evaluated for chemical composition and in vitro activity against the *C. Albicans*. In vitro analyses were carried out employing NEs with OEO, carvacrol and unformulated essential oil. Formulated CAR and OEO resulted in a lower MIC than pure OEO (Fig.1). Positively charge seems not be crucial to the interaction of NEs with the fungi surface: obtained results showed in fact that negatively charged ONEs had the same antifungal activity of the positively charged one. We accept that there is not a real benefit of the positive charge in the fungicide effect. The results obtained evidence efficacy of soy-PC and charged non-phospholipid surfactants in producing stable oil-water NEs by a low-energy emulsification method. The nanoencapsulated OEO and CAR have a better inhibition activity against the fungi than non-formulated OEO, also when negatively charged NEs were used. Formulations have proved to could act as promising alternative green biocides.

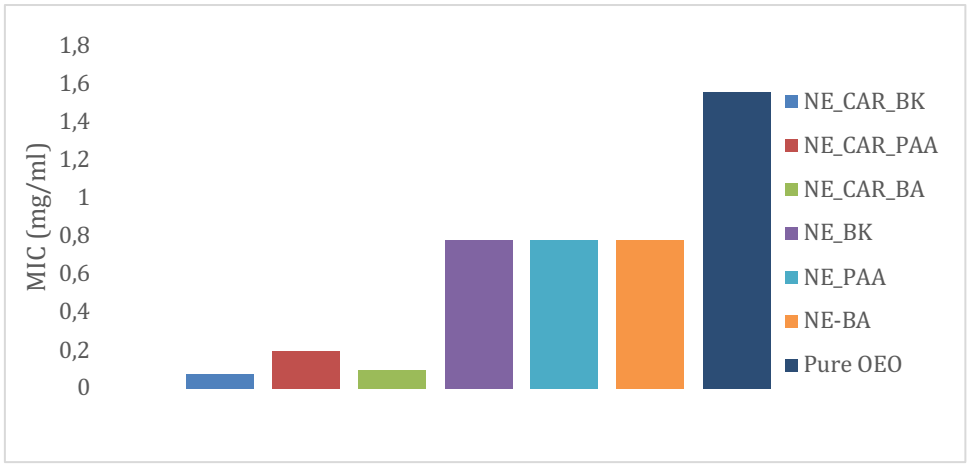

Fig 1. MIC analysis on pure oil, ONEs and CAR-NEs.

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Nanocomposite In situ forming Hyaluronic Acid Hydrogels as potential bone filler for the regeneration of irregular bone defects

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Keywords: Bone Filling, Injectable nanocomposite hydrogels, Osteogenic potential, Hyaluronic Acid, Accelerated Bone Regeneration

Bone defects that arise from congenital anomalies or high-incidence diseases, such as osteomyelitis, osteosarcoma and osteoporosis, represent a major clinical challenge. Long-term treatments cause significant harm to the human body leading to substantial healthcare costs worldwide. [1] Currently, orthopedic and dental implants made of metal or bioceramics provide mechanical strength comparable to bone tissue. However, their limited flexibility, adaptability, and biodegradability hinder patient mobility and natural tissue regeneration. [2,3] In situ-forming hydrogels based on biopolymers emerge as ideal alternative for irregular bone defects treatment due to their biodegradability, extracellular matrix-like structure, adaptability to the implantation site, and ability to recruit nutrients essential for cell proliferation. [1, 4, 5] These systems can be loaded with osteoinductive substances like hydroxyapatite (HAp) [2, 6], calcium phosphate [7], β-tricalcium phosphate [8], and magnesium particles (MgMPs) [9, 10] to accelerate the regeneration process. Among the most used biopolymers, hyaluronic acid (HA) is highly regarded in tissue engineering for its versatility, ability to promote cell proliferation, involvement in multiple biosignaling pathways, and ease of chemical modification to achieve desired properties. [3, 11, 12, 13, 14] In this study, we utilized UV photopolymerization based thiol-yne click chemistry (TYC) to develop HA-based, nHAp-loaded, covalently crosslinked hydrogel networks as bone fillers for the treatment of irregular bone defects. Alkyne modified HA was crosslinked with a trifunctional thiol crosslinker using two different molar ratios between thiol groups and the alkyne-functionalized polysaccharide backbone. The presence of varying amounts of nHAp in the gelling solution did not negatively impact gelation time or hydrogel stability. Furthermore, the incorporation of nanoparticulate bioactive ceramic reduced the swelling ratio of the nanocomposite hydrogels while enhancing their compressive modulus, confirming the positive effect of the doping agent on the physicochemical and mechanical properties of the materials. Ex vivo studies on bone specimens demonstrated the feasibility of the proposed injectable bone-filling approach and highlighted the hydrogel's stability upon contact with bone for up to 28 days. In vitro cytocompatibility tests conducted on two different cell lines confirmed the material's safety, while the Alkaline phosphatase activity and Alizarin Red staining suggested its potential osteogenic differentiation.

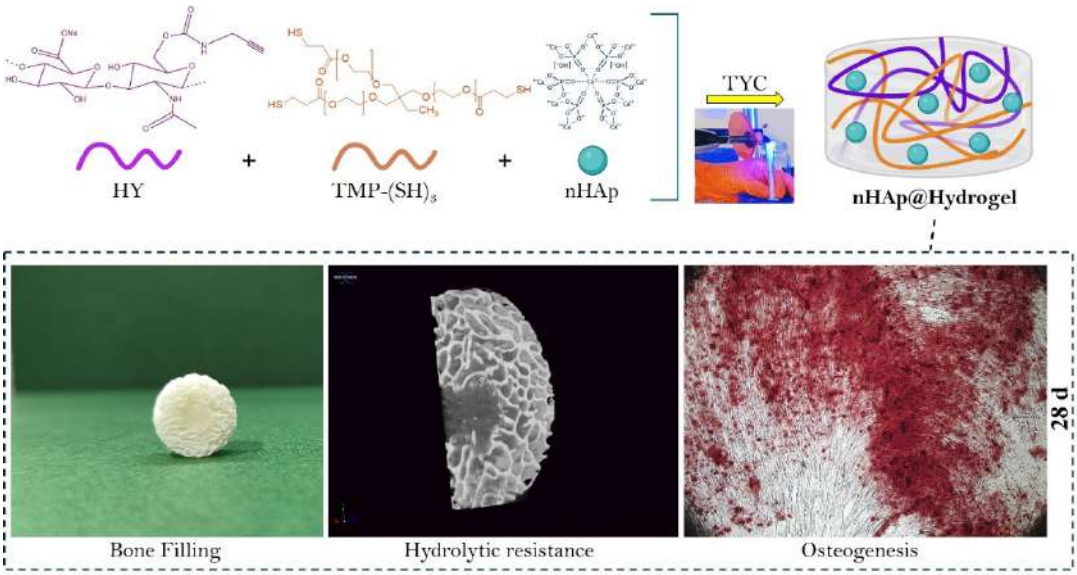

Figure: Preparation of nanocomposite hydrogel based on thiol–yne click chemistry (TYC) and bone filling, hydrolytic degradation and osteogenesis results.

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Noncompetitive αAmylase and αGlucosidase Inhibitors for Type 2 Diabetes Treatment

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Keywords: type 2 diabetes, noncompetitive inhibitors, α-amylase, α-Glucosidase.

α-Amylase and α-glucosidase are crucial targets for antidiabetic therapies. These two enzymes play key roles in saccharide hydrolysis, directly contributing to elevated blood sugar levels. The inhibition of these enzymes could delay glucose absorption and enhance its regulation in type 2 diabetes, leading to significant improvements in quality of life and potentially increasing life expectancy for patients. [1,2,3] We synthesized a library of novel nicotinic acid derivatives using an innovative green synthetic approach and evaluated their inhibitory activity against α-amylase and α-glucosidase. Two compounds, named compound 8 and compound 44, were found to inhibit α-amylase with IC_{50} of 20.5 and 58.1 μM, respectively. Notably, compound 44 exhibited a remarkable inactivation level, surpassing the efficacy of the control compound, acarbose. Additionally, two other molecules, compound 35 and compound 39, demonstrated inhibition against α-glucosidase, with IC_{50} of 32.9 and 26.4 μM, respectively, both showing a significant improvement in enzyme inactivation compared to the control. Mechanistic studies revealed a noncompetitive inhibition mechanism for both α-amylase and α-glucosidase, offering advantages over competitive inhibitors. Furthermore, compounds 8 and 39 exhibited the best IC_{50} values among noncompetitive inhibitors reported in the literature [4], suggesting potential for developing more effective hypoglycaemic agents for type 2 diabetes treatment.

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Targeting the FLT3 receptor in AML therapy with Pharmacophore-Based Virtual Screening: An *in silico* approach to discovering new potential inhibitors

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Keywords: Acute Myeloid Leukemia, FLT3 receptor, type II inhibitors, pharmacophores, virtual screening.

Acute Myeloid Leukemia (AML) is an aggressive form of myeloid blood cell cancer, often associated with low survival rates. In recent years, increasing evidence have demonstrated that the activation and mutation of FLT3 receptor is closely implicated in the occurrence and low response to therapy of AML. [1] Therefore, FLT3 represents an attractive therapeutic target. Quizartinib (**1**, Figure 1) is a second generation FLT3 inhibitor of type II, with high selectivity and antiproliferative activity (IC_{50} = 0.56 nM). Due to serious adverse effects emerged in clinical trials, the identification of novel FLT3 inhibitors is an ongoing challenge, and - within this framework - our research group has recently identified a novel family of compounds (**2**, Figure 1) from which potent candidates emerged (IC_{50} = 0.0011-0.578 μM). [2] With the aim of identifying potential new candidates as FLT3 inhibitors, by using pharmacophore approach to analyse the molecular functional features needed for the target-ligand binding [3], a thorough computational investigation at the University of Vienna, in cooperation with the Cheminformatics Group, has been carried out. Four pharmacophores were developed and optimized for FLT3 (**3**, Figure 1) to perform a Pharmacophore-Based Virtual Screening on almost 120 million small molecules from various commercial collections. The final selection of hits was conducted based on different criteria, including compatibility with the pharmacophore features, solubility and cardiotoxicity. Moreover, MD simulation was performed to further analyse the potential new hits. The most promising compounds will be submitted to biological assays to evaluate FLT3 kinase inhibitory activity, and the structure of the best hits will be further modified by rounds of chemical synthesis to obtain optimized compounds in terms of potency and efficacy.

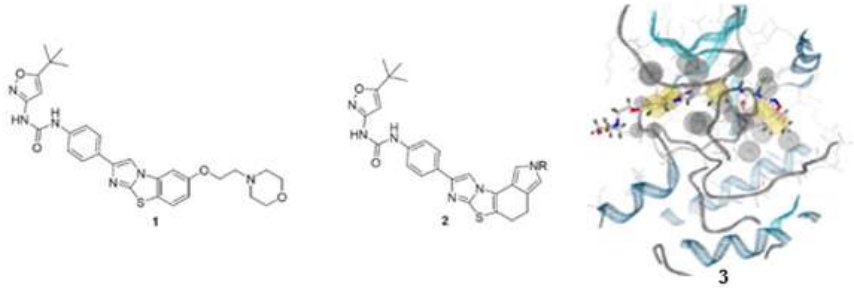

Figure 1. 1 Quizartinib; 2 Novel family of tetracyclic compounds; 3 Crystal structure of Quizartinib in complex with FLT3 (pdb code: 4RT7), LigandScout 4.5 suite (Inte:Ligand GmbH). [4]

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Platforms for therapeutic RNA/DNA delivery: Fusogenic ANANAS-based system for targeted delivery of siRNA/miRNA

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Keywords: gene delivery, nanomedicine, nanoparticles, targeted therapy

Introduction. The ideal formulation to deliver RNA therapeutics should: 1) protect the RNA cargo from RNAse degradation; 2) possess the necessary cell trafficking and fusogenic properties to allow the delivery of nucleic acid (NA) to the cell cytoplasm, and 3) be capable of inducing the biological response only at the target cell/tissue.
Currently, the most efficient NA delivery systems rely on viral vectors or synthetic lipid-based formulations that possess only two of these characteristics, since they cannot provide selective targeting. [1] In this work, we evaluated the potentials of Avidin-Nucleic-Acid-NanoASsemblies (ANANAS) as a platform for RNA delivery. ANANAS are nanosized colloidally stable soft polyavidin (350 avidins/NP) toroids ideally suited to explore multifunctionality. [2]vANANAS nanoparticles are formulated to a) selectively target cells of interest due to specific targeting elements (e.g. Cetuximab for EGFR+ tumor) and promote internalization; b) protect RNAs cargo from RNAse degradation because of surface PEGylation; c) deliver intact RNAs to the cytoplasm thanks to lysosomal escaping strategies and reversible anchoring of the cargo (disulfide bond in the linker between biotin and RNAs) (Figure 1). H1299-eGFP cells have been used to study the silencing efficiency of the formulated ANANAS system. To this end, anti-GFP siRNA was loaded onto targeted and fusogenic ANANAS nanoparticles and different analysis have been conducted to evaluate the formulation stability and the silencing effect on GFP+ cells. Methods. Pre-formulation studies were performed by mixing, with stoichiometric control, core ANANAS with different biotin moieties, namely Cetuximab antibody to target EGFP+ cells, endo/lysosome escape moieties and anti-GFP siRNA.Acrylamide electrophoresis and FPLC analysis were carried out to assess the maximum loading capability of the biotinylated moieties and the stability of the NA, alongside DLS analysis of the final formulation. FACS analysis and *in vitro* fluorescent confocal microscopy studies were carried out on eGFP-H1299 cells to study the internalization of nanoparticles and their localization within cells and, finally, the silencing efficacy of the selected formulations was tested by cytofluorimetric analysis. Results. The results indicate that the ANANAS platform can be a valuable system to deliver siRNAs, as they can be easily loaded into a targeted and fusogenic system. The formulation with all the biotinylated elements (siRNA, Cetuximab and fusogenic) is colloidally stable and Cetuximab-driven NPs successfully interact and internalize in H1299 cells. However, no silencing in the GFP expression was seen by cytofluorimetric analysis, even if the pre-formulations studies were overall promising. Further studies will be conducted to upgrade the existing ANANAS formulation.

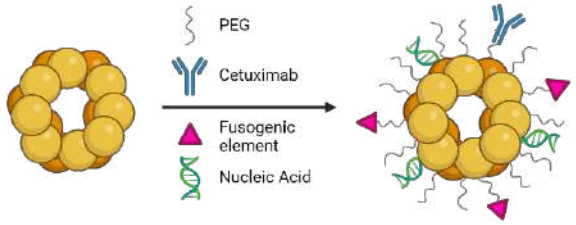

Figure 1. Composition of ANANAS nanoparticles formulation with PEG coating, the targeting agent (Cetuximab antibody), the fusogenic element and the nucleic acid.

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Antisense Oligonucleotides as a Therapeutic Strategy in Rare Genetic Diseases: Applications in Hutchinson-Gilford Progeria Syndrome and Duchenne Muscular Dystrophy

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Keywords: Genetic disease, Genetic mutation, antisense oligonucleotides therapy, Progeria, Duchenne Muscular Dystrophy.

Rare genetic diseases represent an important challenge for medicine today. In recent years, various gene therapy strategies have been explored with the goal of correcting the defects underlying these diseases, but many still lack effective treatments. Among the most promising approaches is the use of antisense oligonucleotides (ASOs), short nucleotide sequences that can specifically bind to mRNA and modulate its function. Thanks to targeted chemical modifications, ASOs can be designed to interfere with splicing, promoting or blocking the inclusion of specific exons, thus restoring proper expression of the mutated gene. Our work aims to evaluate the activity of ASOs specifically designed for two monogenic diseases: Hutchinson-Gilford Progeria Syndrome (HGPS) and Duchenne Muscular Dystrophy (DMD). HGPS is caused by a point mutation in exon 11 of the LMNA gene (c.1824C>T), which activates a cryptic splicing site and leads to the production of progerin, a toxic form of lamin A. Progerin induces alterations in nuclear morphology, oxidative stress, premature senescence, and genomic instability (Gonzalo S. et al., 2016). We aim to evaluate ASOs that can mask the aberrant splicing site and promote the expression of the correct isoform, testing them in engineered HCT116 cells expressing the mutated form of the LMNA gene (cDNA) and in patient fibroblasts. The efficacy is assessed by RT-qPCR, Western blot, immunofluorescence analysis, and studies of cellular senescence markers. DMD is caused by mutations in the DMD gene, including stop codon mutations, which lead to premature translation termination and the absence of dystrophin. This results in severe muscle wasting, respiratory and cardiac failure, and even death before the age of 30 (Falzarano et al., 2015). Specific Readthrough-ASOs (R-ASOs) are designed to bind to the region immediately downstream of the stop codon to promote ribosomal readthrough. The ultimate goal is to identify effective molecules that can restore gene expression and protein function, creating new therapeutic perspectives for diseases that currently have non curative solutions.

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Electrolyte-Gated Organic Transistors for Electrically Monitoring the Aggregation of Oligoglycine Tectomers

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Keywords: electrolyte-gated organic field effect transistors**,** tectomers, self-assembly, biosensensing

Oligoglycine refers to short chains of glycine amino acids that self-assemble into larger supramolecular structures called tectomers. These tectomers exhibit unique physicochemical properties due to their pH-responsive behavior and surface functional groups, enabling diverse applications in materials science and biotechnology. Oligoglycine tectomers are known to form self-assembled monolayers (SAMs) on metal surfaces. However, determining the critical concentration for monolayer formation is not straightforward. Electrolyte-gated organic field-effect transistors (EGOFETs) are low-cost electronic devices that show high sensitivity and capability to recognise changes at their electrolyte/ gate electrode and electrolyte/organic semiconductor interfaces. Hence EGOFET are highly attractive for the development of biosensing platforms. Further, these devices have also been applied to monitor dynamic processes, such as the aggregation of amyloid proteind and surfactants. In this work, we show the EGOFETs are also a promising tool to study the formation of tectomer SAMs on gold. A shift in the EGOFET threshold voltage is observed in the devices when the tectomer SAM is formed, which is caused by a modulation on the Au work function. In addition, future studies on oligoglycine's pH-responsive assembly which are known to influence the SAM compactness via hydrogen bonding and electrostatic interactions could lead to interesting insights for applications such as drug loading and release.

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Assessing biomarkers in biological fluids by combining microfluidics and organic electrochemical transistors

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Keywords: biosensors, surface functionalization, microfluidics, blood, organic electrochemical transistors

Organic Electrochemical Transistors (OECTs) have recently demonstrated breakthrough capabilities towards biomedical applications opening new avenues towards nanosensing. [1,2] These sensors work as amplifying transducers of biochemical signals, thereby representing a unique tool to detect molecular species at very low concentrations that are hardly to track experimentally. In addition, recent progress in microfluidics and biosensing technologies has opened avenues for the creation of compact and effective diagnostic instruments. This study introduces an innovative integrated lab-on-a-chip apparatus coupled with electrochemical transistors for the precise analysis of crucial biomarkers in biological fluids, ensuring both high sensitivity and specificity. Electrochemical transistors, known as OECTs, are under thorough investigation due to their capacity to enhance ionic-electronic transduction, holding substantial promise for biosensing tasks. [2,3,4,5,6,7] These devices boast a relatively simple design, comprising three electrodes—source, drain, and gate—alongside an active layer of conductive polymer, specifically PEDOT:PSS. The polymer channel links the source and drain contacts and is immersed in an electrolyte, along with the gate electrode. By applying a voltage (V_{GS}) to the gate, ions migrate from the electrolyte into the polymer channel, effectively modulating the electronic current (I_{DS}) between the drain and source electrodes in a reversible manner. Importantly, these devices operate at voltages below 1 V, making them compatible with interactions with biological fluids without causing significant hydrolysis or molecular denaturation. The experimental methodology involves examining OECT responses under various operational conditions such as electrolyte composition, applied voltage, and device structure in fluids like human blood, seminal liquid and buffer solutions with proteins. Furthermore, the study investigates the influence of surface alterations and biofunctionalization on sensor performance. [3,4] Through systematic experimentation and analysis, this study focuses on investigating the response of OECTs when employed for biosensing purposes; in particular the performance of OECTs in detecting biomolecules and analytes present in biological samples is studied. The results obtained shed light on the feasibility and potential limitations of utilizing OECTs for biosensing applications, providing valuable insights for further optimization and development in the field of bioelectronics.

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Celecoxib activates a ROS-mediated mitochondrial apoptotic program in 2D and 3D melanoma cell cultures

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Keywords: Cutaneous Melanoma, Celecoxib, 2D and 3D cell cultures.

Cutaneous Melanoma is the most aggressive type of skin cancer characterized by high mortality rates due to metastasis development and therapy resistance. [1] Therefore, new therapeutic strategies need to be found. Inflammation is one of the key factors in melanoma progression, as it promotes tumor growth, metastasis and therapy escape. [2] One possible approach to target inflammation is using non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzymes and reduce prostaglandin synthesis. [3] Previously, we demonstrated that celecoxib, a COX-2 selective NSAID, exerts cytotoxic, anti-migratory and anti-invasive effects in two-dimensional (2D) and three-dimensional (3D) cell cultures of A2058 and SAN melanoma cell lines. [4]
In this study, we further investigated the molecular mechanisms underlying celecoxib-induced cytotoxicity. To this end, we analyzed the effects of drug treatment using FACS, fluorometric assays, western blot, and immunofluorescence. We found that celecoxib activated an apoptotic program without inducing necrosis, as extracellular LDH levels remained largely unchanged. Furthermore, ROS levels and p53 protein expression increased. Although BAX levels remained unchanged and BCL-2 increased, mitochondrial membrane depolarized, and cytochrome c leaked out after treatment. Additionally, SOD-2 protein expression decreased in 2D cell cultures, whereas, in spheroids, it increased in A2058 and remained unchanged in SAN. These findings suggest that celecoxib induces apoptosis through a mitochondrial pathway that does not depend on the BAX/BCL-2 ratio. Indeed, BCL-2 upregulation may represent a compensatory mechanism that is insufficient to prevent cell death, possibly because other pro-apoptotic proteins are involved, such as those of the BH3-only family. The activation of apoptosis could be ROS-mediated and the reduction of SOD-2, observed in 2D cultures, suggests a decreased cell tolerance to ROS. Overall, our study provides novel insights into the molecular mechanisms of celecoxib-induced cell death in melanoma, highlighting its potential as an anti-cancer agent.

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Molecular Dynamics-Guided Design of Self-Assembling Antimicrobial Peptides

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Keywords: peptide, nanostructure, molecular dynamics, antimicrobial, antibiofilm

Antimicrobial resistance (AMR) is one of the most significant global health challenges of the 21st century, exacerbated by the excessive use of antibiotics and the limited discovery of novel antimicrobial agents. In this scenario, antimicrobial peptides (AMPs) have emerged as promising alternatives, with over than 5000 entries in the Antimicrobial Peptide Database (APD). [1] However, their clinical translation faces several limitations, including their high production costs, cytotoxicity and poor stability under physiological conditions.

Our work focuses on designing novel short self-assembling peptides with tailored antimicrobial and/or antibiofilm activity. Aside from their ability to interact with bacterial barriers, their supramolecular organization allows for the development of systems with improved stability and stimuli-responsiveness. [2] We are investigating different types of nanostructures to elucidate Structure-Activity Relationship (SAR) between supramolecular organization and antimicrobial efficacy.

The development of our peptide library is guided by coarse-grained molecular dynamics (CG-MD) simulations using the GROMACS 2023 package, following established protocols. [3] These simulations allow us to predict aggregation propensity.

The most promising AMPs candidates will be further investigated to assess their efficacy and toxicity profiles *in vitro* against various types of bacterial strains and eukaryotic cells. [4,5] To enhance evaluation accuracy, we are developing advanced bioengineered platforms, including 3D biofilm infection models and co-cultures of eukaryotic and prokaryotic cells, to understand potential resistance mechanisms and evolutionary adaptations.

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Rational Design and Synthesis of Novel Alkylamides Based on Natural Leads from *Zanthoxylum* spp. and *Acmella oleracea* and Evaluation of Their Biological Activity

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Keywords: Alkylamides, antimicrobial activity, Analgesic agents, structure-based drug design

Zanthoxylum spp. and *Acmella oleracea (janbu)* have long been recognized as important medicinal plants across various cultures. Their range of applications in traditional medicine is wide and includes treatment of infections and chronic pain disease, in this regard several studies have highlighted the correlation of potency of their activity with the presence of alkylamides, a class of secondary metabolites, also known as fatty acid amides. [1,2] They are characterized by an amide group with a saturated or unsaturated hydrocarbon chain on the acyl moiety and aliphatic, cyclic, or aromatic groups bounded to the nitrogen atom. [3] This study reports the design, synthesis, and pharmacological evaluation of novel alkylamide derivatives in order to expand the structure–activity relationships of this class in the identification of new lead compound derivative as promising analgesic and antimicrobial agents.

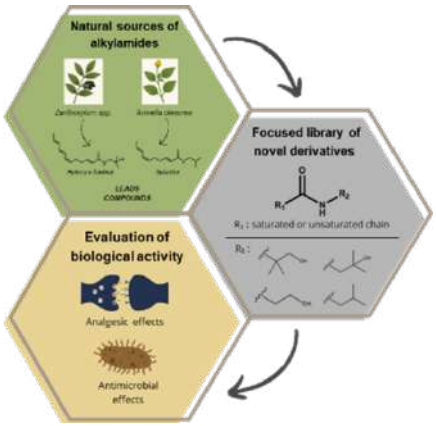

Figure. Rational design, synthesis, and biological evaluation of novel alkylamide derivatives resulting from natural leads of *Zanthoxylum* spp. and *Acmella oleracea.*

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Selection and characterization of human single-chain antibody fragments capable of recognizing therapeutic anti-sense oligonucleotides bearing phosphorothioate backbones

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Keywords: phage display, anti-sense oligonucleotides, scFv

There is a pressing need for rapid and efficient methods to follow the distribution of anti-sense oligonucleotides and other RNA therapy drugs in tissues and subcellular compartments, after their administration. These methods are crucial to develop strategies to efficiently deliver RNA therapy drugs to their intended targets. We are using phage display libraries to isolate specific antibody fragments (scFv or single chain Fragment variable) capable of binding to epitopes associated with the phosphoro-thioester (PS) backbone present in almost all antisense oligonucleotides. To this end, we have developed a strategy involving the preliminary elimination of unwanted specificities by negative selection using phosphodiester (PO) oligos as bait, followed by positive selection on PS oligos. After three selection rounds, we have significantly enriched our libraries for antibody-binding clones and are testing individual clones for specific binding. In parallel, we have successfully amplified from selected and unselected phage display libraries CDR3 heavy chain region using next-generation sequencing. By this technique, we have detected marked reductions in library diversity, concomitantly with increases in individual clone copy numbers, consistent with successful enrichment of the libraries for specific clones.
We have thus far isolated several clones with the following properties: 1) efficient binding to PS oligos containing either sequence targeting a conserved region of the ZIKA virus (ZIKV) genome or a scrambled sequence, indicating that the isolated clones are not sequence-specific; 2) lack of reactivity with a range of ASOs, in which all individual nucleotides are linked through PO links, including a PO oligonucleotide with the above mention ZIKV sequence. We are currently trying to improve the affinity of these clones (as assed by competition ELISA) by using an approach in which synthetic CDR3 sequences are constructed using codons that were found -using next generation sequences- to be particularly enriched at certain amino acid positions during the selection process. Overall, this approach should lead to the isolation of high-affinity clones, suitable for efficient binding of a wide variety of ASO bearing PS backbones.

Nanoparticles for pulmonary drug delivery: MALDI-TOF analysis of imatinib-loaded liposomes after intranasal administration in a murine model of pulmonary fibrosis

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Keywords: Pulmonary Drug Delivery, Nanomedicine, Pulmonary Fibrosis, Liposome

Pulmonary fibrosis is a progressive lung disease associated with a poor survival. It is characterized by irreversible fibrotic deposition and injury of the lung parenchyma, leading to severe functional impairment. The pathogenesis is multifactorial and involves several mechanisms. [1] The current therapies are still poorly effective and the development of novel therapeutic drug treatments is urgently needed. [2] Nasal instillation of nanoformulations is an attractive alternative. However, its clinical application remains difficult. [3] The development of an analytical platform able to determine the fate and safety of nanoparticles is crucial. The first aim of the study was to test the ability of biocompatible liposomes to reach the pulmonary target after intranasal administration. Biodistribution of nanocarriers was investigated *ex vivo* in bleomycin-treated mice after labelling of liposomes with a fluorescent dye. Histopathological analyses were performed to test both inflammation and fibrosis. The evaluation of liposomes ability to reach lung parenchyma demonstrated that liposomes distributing in the lungs with high concentration and avoid an off-spread to the off-target organs. In the second part of the study, diseased mice were treated with two types of nanocarriers: liposomes and hyaluronic acid-decorated liposomes, both loaded with imatinib. Spatial distribution of analytes and drug quantification in tissues were performed using MALDI time-of-flight mass spectrometry. MALDI drug analysis in lung tissue sections highlighted that imatinib loaded into liposomes reached lung after intranasal administration and drug signal persisted in the parenchyma up to six hours post administration. Drug signal in lung free imatinib-treated animals was detectable only for two hours post-administration. MALDI analysis was also conducted on kidney tissue sections. Two hours post-administration high signal of drug was observed in the kidneys of animals treated with free imatinib. In contrast, no imatinib signal was detected in animal treated with either of the two liposome formulations at the same time points. This work established that liposomes represented a promising drug delivery system approach for lung targeting. Furthermore, loading imatinib into liposomes improved its half-life in the lung and reduced drug off-spread, at least during the first time points. No significant differences in drug distribution and deposition were observed between decorated with hyaluronic acid or nude liposomes in either the lung or the kidney parenchyma. Functionalization of liposomes with hyaluronic acid does not appear to positively influence drug persistence in the lung. A quantitative study of drug concentration in the organs and plasma will be performed, along with an investigation into potential interactions between pulmonary cells and the nanocarriers.

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Identification of new small molecules as F508del-CFTR correctors for the treatment of cystic fibrosis.

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Keywords: cystic fibrosis, CFTR correctors, F508del-CFTR, tricyclic core, drug discovery

Cystic fibrosis (CF) is a genetic disease caused by mutations that impair the function of the CFTR chloride channel. To correct the basic defect, various pharmacological agents, named CFTR modulators, have been developed. Such agents can stabilize misfolded protein and increase membrane expression (CFTR correctors) or restore channel opening (CFTR potentiators). [1,2,3] Although significant advances have been obtained by combinations of correctors with complementary mechanism, new pharmacological modulators are still needed to maximize the rescue of F508del and other CFTR mutants with trafficking defects. Supported by Italian Cystic Fibrosis Foundation, we have identified a novel family of small molecules (PP compounds), as correctors with high efficacy in the rescue of F508del-CFTR on native epithelial cells of CF patients particularly in combination with class 1 correctors (VX-809). [4] We developed a library of compounds based on a tricyclic core (PP) with the main objective to obtain a lead compound endowed with improved activity as corrector and acceptable drug-like characteristics. The new compounds were functionally tested on CFBE410$^-$ cells expressing F508del-CFTR and the halide-sensitive yellow fluorescent protein (HS-YFP), disclosing results as quenching rate (QR) a parameter reflecting anion transport. The most active compounds were then validated in short-circuit recordings on primary airway epithelial cells (bronchial and/or nasal) and in biochemical and microscopy assays to evaluate CFTR maturation/trafficking. PP028 was found as a lead candidate for the high rescue of F508del-CFTR in primary airway epithelial cells from CF patients, strongly synergizing with VX-809, also indicating that PP compounds act as class 3 correctors, since they induce synergistic effect when combined with class 1 (VX-809) and 2 (3151) correctors but not with class 3 (VX-445, 4172). Thus, by structure-activity relationship (SAR) studies, we started a multiparametric optimization exploring the chemical space around the tricyclic compounds. By iterative cycles of chemical synthesis and functional evaluation we isolated compounds with enhanced features in terms of corrector activity (potency/efficacy) taking into account also the drug-likeness. We recently obtained active compounds by manipulation of a less explored region of the scaffold, prompting further synthesis to better understand the role of this region in chemical interaction to CFTR affecting the corrector activity. In conclusion, the new class of compounds appear promising for the future development of combinatorial treatments for CF. This research is supported by the Italian Cystic Fibrosis Research Foundation (FFC#4/2018, FFC#3/2020 and Molecules 3.0). We acknowledge MUR (PNRR-M4C2-I1.3 Project PE_00000019 "HEAL ITALIA").

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Albumin conformational changes due to oxidation processes: a systematic approach

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Keywords: Human Serum Albumin, oxidative stress, protein oxidation, conformational change, systematic approach.

Human serum albumin (HSA) is a 66.5 kDa, non-glycosylated, globular and monomeric protein. [1] It represents the most abundant protein in the human serum and it is involved in different functions, such as oncotic pressure regulation, antioxidant activity, drug, fatty acids, metal ions and hormones binding and transport. [2] Moreover, many studies report its capability to bind the amyloid β peptide and inhibit its aggregation. [3] However, these features can be affected by conformational changes due to oxidation processes related to aging or pathological conditions such as diabetes, heart, kidney and neurodegenerative diseases. Interesting, a higher amount of oxidated HSA can be found in the cerebrospinal fluid of Alzheimer's disease patients. [4,5]
In this work a systematic approach was used to study and compare oxidative damages and conformational changes due to artificially induced oxidation procedures form the literature with Chloramine T and via the Fenton reaction on the commercial recombinant HSA isolated from *Oryza Sativa/rice* (OrsHSA). Results showed that the same two oxidation procedures lead to different damages and conformational changes despite the same amount of oxidative equivalents were used. These differences were reflected in different absorption and fluorescence spectra, binding capability of the 8-Anilino-1-naphthalenesulfonic acid (ANS) fluorophore and hydrodynamic radius. Moreover, OrsHSA before and after oxidation was compared to commercial HSA isolated from blood (bHSA). This comparison indicated that bHSA is partly oxidatively damaged. Overall, the results suggest that oxidative damaged HSA can undergo conformational changes which could impact its interactions with binding partners and compromise its function.

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Pyro-electrohydrodynamic approach for a high-sensitive and efficient jet printing of biomolecules

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Keywords: detection, neurodegenerative disease, pyro-electrohydrodynamic jet, low abundant samples

Biomolecule printing is advancing biomedical research, drug discovery, biosensor fabrication, and diagnostics. Among emerging techniques, pyro-electrohydrodynamic jetting (p-jet) stands out for its precision in depositing biomolecules into microscale spots with high reproducibility. Utilizing the pyroelectric effect of lithium niobate, p-jet generates ultra-small droplets (down to 1 pL) [1], forming spots between 3 and 15 μm in diameter, surpassing conventional pin-contact printing. [2] MATLAB analysis confirms the stable dynamics of micro-droplet expulsion. This method enhances protein microarrays by improving sensitivity and specificity, enabling detection of low-abundance proteins at picogram-per-milliliter levels. It is particularly valuable for early detection of neurodegenerative disease markers like Tau protein, α-synuclein, and β-amyloid. Studies demonstrate its reliability through immunodetection experiments, including BSA immobilization and diverse binding strategies [3]. Remarkably, p-jet has enabled the first detection of pre-aggregated Tau in artificial urine at femtomolar concentrations (LOD = 130 fM) and identified low-abundant Aβ1-42 aggregates in a simulated body fluid.
The compact, cost-effective p-jet system facilitates the development of miniaturized diagnostic devices, holding great promise for point-of-care applications and continuous health monitoring. Its potential for managing various diseases and improving patient outcomes positions it as a transformative tool in biomedical diagnostics.

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Ferritin-polyamine conjugates for targeted delivery of siRNA therapeutics

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Keywords: polyamines, oligonucleotides, ferritin, bioconjugates, RNA therapeutics

Small interfering RNA (siRNA) has emerged as a powerful tool for gene silencing, offering significant therapeutic potential for treating various diseases. However, the clinical success of nucleic acid-based therapies depends on developing effective delivery systems, which face significant challenges such as targeted delivery, stability, and efficient cellular uptake. To overcome these limitations, a "humanized" chimeric Archaeal ferritin (HumAfFt) was engineered as a nanocarrier to efficiently transport negatively charged siRNA duplexes. In a previous work, to enhance siRNA electrostatic loading and subsequent cellular delivery, the internal cavity of HumAfFt was functionalized with cyclic polyamines (PAs), which were conjugated to strategically placed cysteine residues inside the HumAfFt through chemoselective thiol-coupling. The research activity aimed to develop a more efficient ferritin-based delivery system to improve RNA encapsulation via *electrostatic interactions* and, thus, RNA release from endosomal compartments. Herein, our strategy envisages the creation of a synthetic library of poly-cationic molecules to establish the best relationship between the structure of positively charged linkers and negatively charged oligonucleotides and the expected interaction (namely, the structure-interaction relationship approach). Accordingly, novel linear polyamine linkers were designed and synthesized, namely R-PA-3.2. The synthetic approach relies on a straightforward strategy where iterative Steglich reactions couple the carboxylic groups of *N*-protected amino acids to the amino group of the main chain. The key building blocks have been identified in methylamine, β-alanine, and glycine in different ratios. Based on previous polycationic linkers, these novel compounds were decorated with a pentafluorobenzenesulfonamide moiety for the selective bioconjugation of cysteine residues inside the protein inner cavity (figure 1). [1,2]

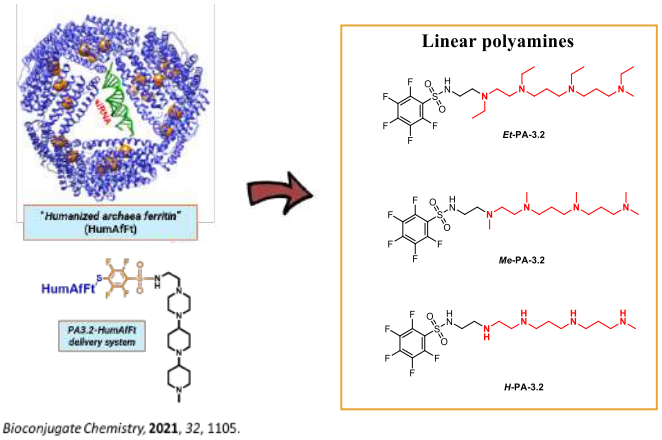

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Figure 1. Encapsulation of small oligonucleotides via the functionalization of the internal cavity with polyamines.

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Development of a Chemo-Assisted Optical Device for Serum Albumin Sensing

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Keywords: Albumin; PAD; Sulfonephthaleins; Colorimetric; Chemometrics.

Albumin serves as a biomarker for kidney and liver diseases, cardiovascular risks, and nutritional status. [1] Diagnostic quantification methods include chromatographic [2], colorimetric [3], immunological [4], and electrophoretic techniques [5] but recent innovations are moving towards developing Point-of-Care (PoC) albumin sensors that are cost-effective and user-friendly and allow an early diagnosis or monitoring at home. [6] This study presents a paper-based colorimetric sensor capable of qualitatively detecting albumin in aqueous solutions at concentrations down to 0.5 µM. Colour Catcher® (CC®) was selected as a solid support due to its affordability and ion exchange properties that allow albumin sensitive dyes sorption. [7, 8] The sensing mechanism is based on the metachromasia effect, *i.e.* the color transition of sulfonephthaleins, a class of acid-base indicators, upon albumin interaction in a pH range just below the pH-dependent color turning. [9, 10, 11] While most literature studies follow the OVAT approach (One Variable At Time) for sensors development, the optimization of the sensor, based on bromocresol green (BCG) and chlorophenol red (CPR), was achieved coupling Design of Experiments (DOE) and Principal Component Analysis (PCA). Two colorimetric techniques, UV-Vis spectrophotometry and Digital Color Imaging (DCI), were tested for data collection both for experimental conditions and acidification optimization, aiming at ensuring homogeneity and improve detection reliability. The Euclidean Distances between the samples and their blank in PC1 vs PC2 scores plot were used as responses to compute the DoE coefficients for both BCG-CC@ and CPR-CC@ sensors and detection techniques (Figure 1). For Point-of-Care applications, a qualitative analytical response is more than sufficient, if it occurs with high robustness. For this reason, a chemo-assisted qualitative model was developed, including Plackett-Burman design to control and estimate analytical procedure variability and Linear Discriminant Analysis (LDA) or Quadratic Discriminant Analysis (QDA), to distinguish albumin-containing samples from blanks. Cross-validation allowed the identification of best classification models and external dataset validation estimated qualitative detection limits around 0.5–1.0 µM. The proposed sensors, despite their affordability and apparent simplicity, allow a robust recognition of albumin at low concentrations, overcoming quantitative model limitations in terms of cost, time consumption and need of either sophisticated instruments or trained personnel. The BCG-CC@ and CPR-CC@ sensors demonstrated robustness in aqueous matrices, though further studies are needed to enhance deposition techniques for potential quantification and to confirm sensor application in biological matrices and PoC settings to improve clinical usability.

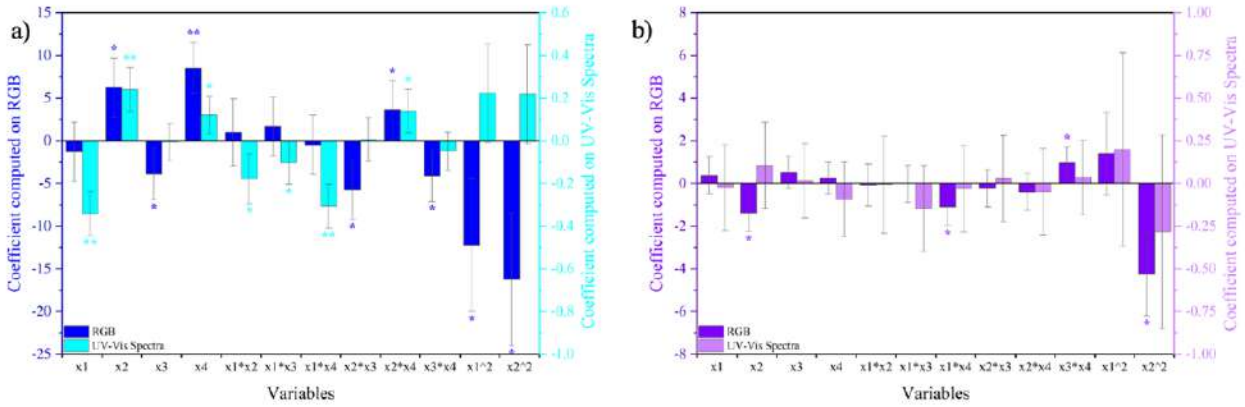

Figure 1: Coefficients plots for the BCG-CC®@ (a) and CPR-CC®@ (b) calculated on ED from scores values in PC1 vs PC2 for both optical techniques.

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Electrochemical sensor to evaluate cellular toxicity through the analysis of nitrite ions

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Keywords: Nitrite, Electrochemical sensors, cell toxicity, Griess

Nitric oxide (NO) and its derivatives, nitrite and nitrate, are involved in various physiological and pathogenic processes. [1] They are found in a wide range of organisms, from humans to plants, fungi, and bacteria. [2] Since nitrite is a stable by-product of NO metabolism, its detection serves as an indirect measure of NO production in cells. [3] NO act as a crucial signalling molecule involved in key cellular functions. It can act as a powerful mediator of cellular damage, leading to toxicity. In humans, NO can contribute to the development of cardiovascular diseases, septic shock under certain conditions [4],cancer [5], hypertension. It also plays an important role in evaluating the inflammatory response, as inflammation can lead to increased production of this molecule. [6] In these contexts, increased levels of NO and nitrite are often associated with cellular damage, oxidative stress, and tissue injury, making their detection crucial for assessing cellular toxicity. The gold standard for nitrite detection in clinical analysis is the Griess method. This colorimetric assay is based on the reaction between nitrites and Griess reagents, producing a colored complex. The spectrophotometric measurement of color intensity provides a direct indication of the nitrite concentration in the sample. Although it is the widely used approach, it is characterized by several limitations such as a time-consuming workflow, the use of multiple reagents and the necessity of a spectrophotometer. However, for clinical applications, electrochemical approaches might offer several advantages in comparison with the traditional existing ones, e.g., Griess method. In fact electroanalysis, and in particular sensors, offer low cost, minimal sample preparation, portability and user-friendly operation. [7] To this context, here we propose the development of a cost-effective electrochemical sensor, printed on flexible polyester substrate, for the detection of nitrites in supernatants of lipopolysaccharide (LPS)-stimulated macrophage cell lines. The sensor successfully detected nitrites at micromolar concentrations, demonstrating performance comparable to gold-standard methods like the Griess assay, without requiring extensive modification steps. These promising results and their good repeatability highlight the potential of the proposed electrochemical sensor as a valuable tool for assessing cellular toxicity and the impact of NO-mediated damage in cells. Due to its low cost, simple design, and portability, it is highly suitable for widespread use in clinical settings, enabling enhanced real-time monitoring of cellular activity and toxicity.

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DNA Condensates as a Platform for Biosensing and Therapeutic Delivery

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Keywords: DNA Condensates, LLPS, Biosensing, Therapeutic Delivery

Membraneless organelles, such as DNA/RNA condensates formed via liquid-liquid phase separation (LLPS), provide a flexible and dynamic platform for biosensing applications in therapeutic advancements. [1,2] The DNA condensates may offer a biomimetic environment for molecular recognition, enabling the detection of disease biomarkers with high sensitivity and specificity. By leveraging multivalent interactions, DNA condensates can be engineered to respond to specific nucleic acid sequences, proteins, or small molecules, making them ideal for diagnostics and targeted drug delivery. [1,2,3] The condensates also play a crucial role in regulating intracellular machinery and maintaining physiological homeostasis in biological system. [2,4] In the context of cancer and infectious diseases, DNA condensate-based biosensors may facilitate real-time monitoring of disease progression and therapeutic response. Additionally, their tunable physicochemical properties may allow for controlled drug release, improving therapeutic efficacy while minimizing off-target effects. [2,3,4,5] Here, we aim to develop nucleic acid condensate as a biosensing and delivery platform of therapeutic for cancer and other critical diseases. Formation of DNA droplets via condensation were confirmed using fluorescence microscopy imaging in the presence of divalent Mg^{2+} and monovalent cations like Na^{+}, or K^{+}. The fluorescence microscopy imaging and dynamic light scattering (DLS) measurement reveal the average size distribution profile of dynamic DNA condensates. Interestingly, the formation and stability of condensates depend on the type of monovalent or divalent ions and the oligonucleotide sequences. This unique property of DNA droplets holds significant potential for biosensing applications and enhanced therapeutic delivery by enabling precise tuning of the local microenvironment. In our next approach, therapeutic oligonucleotides e.g., antimicro RNA are compartmentalized in phase separated droplets to investigate the dynamic behaviour and encapsulation efficiency of DNA condensates for therapeutic cargo in biomimicking microenvironment. As a long-term goal, DNA droplets loaded with therapeutic are intended for delivery into cellular systems to evaluate their real-time cargo release potential, contributing to the development of advanced therapeutic strategies. This work could open a new avenue for targeted delivery of therapeutics in the new era of personalized medicine.

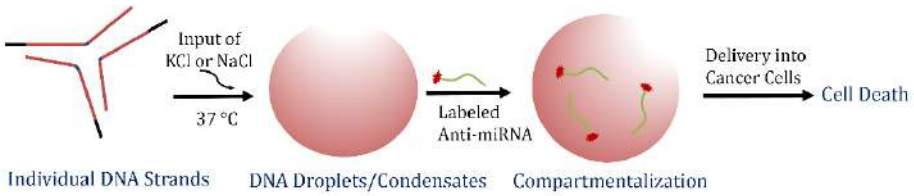

Figure. Schematic representation of DNA condensates as delivery platform of therapeutic.

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Addressing the need for pulmonary siRNA delivery with polyaspartamide-based polyplexes

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Keywords: polyaspartamide, click chemistry, siRNA, polyplexes, pulmonary delivery.

Gene delivery by inhalable nanomedicine is an emerging and promising therapeutic approach for the treatment of several lung diseases, thanks to the ability to complex, protect and transport the genetic material across lung barriers to the cellular target. However, effective pulmonary delivery of siRNA required a carefully engineered delivery system. [1,2] To address this challenge, here, we describe the synthesis of a new α, β-poly(N-2-hydroxyethyl) D, L-aspartamide (PHEA)-based copolymer, PHEA-VS-g-(PMeOX;bAPAE), designed to achieve the pulmonary siRNA delivery via inhalation. Firstly, PHEA was functionalized with divinyl sulfone (DVS) to obtain PHEA-VS, allowing controlled conjugation of 1,2-Bis(3-aminopropylamino) ethane (bAPAE) and poly(2-methyl-2-oxazoline) (PMeOx) at, respectively, 20 mol% and 5 mol% on the total PHEA repeating units. The resulting copolymer exhibited a cationic nature, with protonable amine groups essential for nucleic acid complexation. Additionally, potentiometric titrations confirmed its efficient buffering capacity, while fluorescence microscopy demonstrated pH-dependent membrane destabilization, indicating enhanced endosomal escape potential at low pH values. Gel electrophoresis and dynamic light scattering (DLS) confirmed the stable formation of siRNA polyplexes at low polymer/siRNA weight ratios, with colloidal size (< 50 nm) and positive ζ potential. Stability studies revealed that polyplexes remained stable and functional even in the presence of mucus components such as mucin, protecting siRNA from enzymatic degradation. Biocompatibility assessments on human bronchial epithelial cells (16-HBE) showed excellent cytocompatibility, with over 80% cell viability even at high polymer concentrations. Once the copolymer's complexation ability was assessed, resulting in the formation of stable nanosized polyplexes, these were functionalized on the surface with a cell-penetrating peptide azide (CPP-azide), to enhance the cellular uptake, exploiting the azide-alkyne Huisgen reaction. [3] The functionalization reaction proceeded efficiently for about 80%, resulting in CPP decorated polyplexes with colloidal size and highly cytocompatible. Cellular uptake studies on 16-HBE confirmed efficient polyplex internalization and siRNA release, highlighting the potential of PHEA-VS-g-(PMeOX;bAPAE) as a versatile, effective, and biocompatible siRNA carrier for pulmonary delivery.

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An exploration of FPR2 signaling bias by ureidopropanamide derivatives

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Keywords: neuroinflammation, neurodegenerative diseases, medicinal chemistry, g-protein coupled receptors

Formyl-Peptide Receptor 2 (FPR2) is a G Protein-Coupled Receptor involved in the inflammatory response. This receptor is expressed by immune cells once exposed to pathogens and tissue damage, activating innate immunity and inflammation. In the Central Nervous System, FPR2 is expressed by the microglia, and it is thought to mediate mediating the inflammatory effects of amyloid beta peptides. However Specialized Pro-Resolving Mediators, such as Lipoxin A4 (LXA4), are also active at this receptor, eliciting anti-inflammatory effects. [1,2,3] From the point of view of medicinal chemistry, elucidating the structural determinants of pro-resolving biased FPR2 activation is extremely interesting. [4] Thus, we have chosen to leverage our existing panel of selective FPR2 agonists, featuring a ureidopropanamide scaffold, to investigate this topic. A small set of these derivatives had previously been tested for Ca^{2+} mobilization, β-arrestin recruitment and phosphorylation of ERK1/2 and Akt, showing that structure variations could affect all these pathways. [5,6,7] Here, we present our development of an assay panel that allowed us to extend our studies to a wider range of ureidopropanamide derivatives, and our new insights on the structural determinants of FPR2 biased agonism.

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Selenium Nano-Architectures Selectively Inhibit Cell Viability *in vitro* in HPV-Negative Oral Carcinoma

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Keywords: Selenium, Nano-Architectures, head and neck carcinoma, redox modulation

Selenium (Se) is an essential trace element which exerts diverse physiological activities as component of selenoproteins. [1,2] For example, Se supports the modulation of the redox homeostasis acting as antioxidant at low doses and as pro-oxidant at high doses. [2] Organic and inorganic Se-containing species have attracted attention for biomedical applications because they may play a role in oncology, cardiovascular disease, and Alzheimer's disease. [3] In this context, selenium nanoparticles (SeNPs) have emerged as a safer source of Se, with higher bioavailability and lower toxicity compared to other seleno-compounds. [4] The anticancer potential of SeNPs is associated with the redox modulatory properties of Se and depends on the element concentration in the target site, and its redox state.[2,4] Moreover, SeNPs can exhibit differential activity against malignant and normal cells due to the different reactive oxygen species (ROS) baseline level of the cells.[1,5]

Here, we introduce an innovative source of Se based on biodegradable selenium Nano-Architectures (SeNAs). SeNAs are composed of SeNPs included in a polymeric matrix and surrounded by a silica capsule with an overall size of 150 nm. The synthetic protocol was inspired and adapted from previous NAs including metal nanoparticles, demonstrating high reproducibility and scalability. [6,7] Briefly, spherical SeNPs with an average diameter of 21 nm were synthesized by chemical reduction of selenous acid and embedded in a matrix of poly(ethylene imine) that acts as a template for the formation of a silica shell via a Stöber reaction. SeNAs were characterized by i) transmission electron microscopy and energy-dispersive X-ray spectroscopy to elucidate the morphology and elemental topography, and ii) inductively coupled plasma mass spectrometry to quantify the Se content.

The anticancer potential of SeNAs was verified on HPV-negative head and neck squamous cell carcinoma (HNSCC), a challenging group of malignancies with poor prognosis. This target has been chosen because current treatment for HNSCC relies on cisplatin, a platinum complex burdened by severe side effects. [8] On this regard, a lot of effort is being dedicated to identify cisplatin de-escalation or replacement strategies to reduce off-site toxicity and shift the paradigm of HNSCC management. The cytotoxicity of SeNAs was evaluated *in vitro* on HPV-negative HNSCC cells (SCC-25) and on human bronchial epithelial cells (16HBE140) to evaluate the cancer-to-normal cell selectivity. SeNAs significantly inhibited the proliferation of SCC-25 cells in a dose-dependent manner, with IC50 values in the sub-μM range, without affecting 16HBE140 cells. These preliminary results indicate that SeNAs have an intrinsic selectivity towards HNSCC, highlighting a strong potential as innovative antineoplastic agents for HPV-negative oral carcinoma.

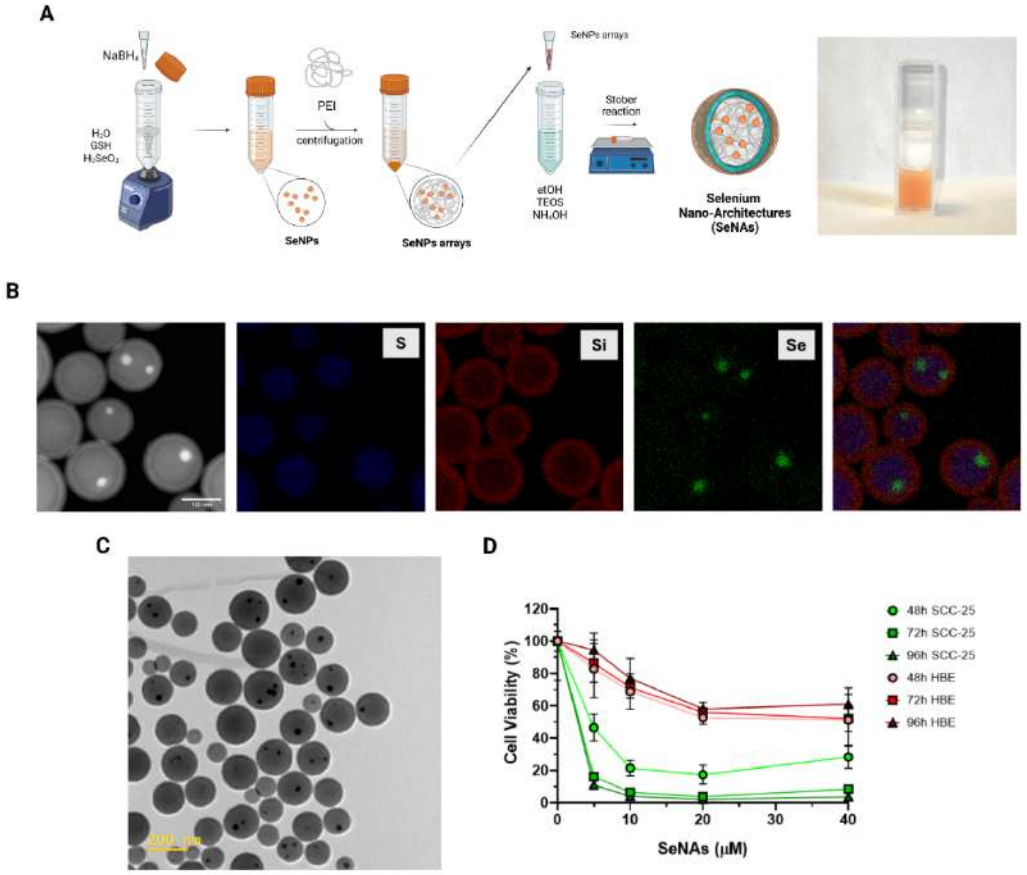

Figure. A) Schematical representation of the synthetic protocol for SeNAs (left), appearance of an ethanol solution of SeNAs (right). B) Scanning transmission electron microscopy image (left) and energy-dispersive X-ray spectroscopy maps of SeNAs. Channels for sulphur, silicon, selenium are shown. Scalebar: 100 nm. C) Transmission electron microscopy image of SeNAs. Scalebar: 200 nm. D) Cell viability (% of control) of malignant SCC-25 and normal 16HBE140 cells at 48, 72 and 96 h after treatment with increasing concentrations of SeNAs.

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Unraveling Water's Influence on Liquid-Liquid Phase Separation

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Keywords: LLPS, water, fluorescence, FLIM, ACDAN

Liquid-liquid phase separation (LLPS) is a fundamental physicochemical process with wide-ranging applications, spanning from biological organization to various engineering fields as for example in biological sensing applications. It occurs when a homogeneous liquid solution demixes into two distinct liquid phases with different compositions, driven by thermodynamic factors such as temperature, solute concentration, and intermolecular interactions. A key but often overlooked aspect of this process is the role of water which may regulate protein phase behaviour through its hydrogen-bonding network by modulating hydration entropy and enthalpy. [1,2]
LLPS of b-lactoglobulin (BLG)-Polyethylene Glycol (PEG) aqueous solutions at neutral pH were studied in different conditions revealing that, in the analysed samples, the solution behaviour is mainly regulated by excluded volume effects. Protein-PEG concentration ratio was found to regulate solution partitioning in two liquid coexisting phases. The combination of spectroscopy and microscopy was exploited to focus on the role of water by means of the environment-sensitive dye ACDAN. The experimental approach enabled the detection of subtle microenvironmental changes associated with water structuring, shedding light on its role in protein phase separation.

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Luminescent Nanomaterial-Enhanced Immunosensing for Personalized Prostate Cancer Biomarker Analysis

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Keywords: *Prostate Cancer, Immunoassay, Nanomaterials, POCT, Biosensing*

Prostate cancer (PCa) is one of the most common malignancies in men, with recent studies indicating that approximately one in fifteen men over the age of 40 develops this disease. [1] The most prevalent type, prostate adenocarcinoma, is often asymptomatic in its early stages and typically present clinical symptoms only when already in an advanced phase. [1,2] Therefore, early screening is crucial for timely and effective diagnosis. To the author's knowledge, currently diagnostic strategies do not meet these requirements as they rely on serum prostate-specific antigen (PSA) levels, which lacks tumor specificity, as elevated levels may also result from benign prostatic conditions. [2, 3] This limitation highlights the need for a panel of tumor-specific biomarkers to improve the accuracy of prostate cancer detection, as well as the development of ultrasensitive immunoassays capable of detecting these markers at low concentrations, typical of early-stage disease. In this work, we aim to create a highly sensitive and specific point-of-care-testing (POCT) device capable of simultaneously detecting multiple PCa biomarkers, including PSA and Prostate-Specific Membrane Antigen (PSMA) in urine and/or serum samples to enable rapid, accessible and reliable screening. [4] We plan to use silica nanoparticles doped with the cationic complex $Ru(bpy)_3^{2+}$, which offer several advantages including enhanced signal intensity—due to the high loading of active dyes—simple synthesis, high stability, and a possible efficient bioconjugation with antibodies exhibiting strong sensitivity and specificity toward our target biomarkers. [5,6] To accomplish a robust protocol applicable to real biological samples, we have started focusing on optimizing the bioconjugation process and evaluating signal enhancement via immunofluorescence microscopy on cell lines with different expression of PSMA and PSA. We are working on an innovative point-of-care testing (PoCT) device with the goal of improving diagnostic accuracy, minimizing false positives, and supporting early detection and personalized treatment strategies in prostate cancer management.

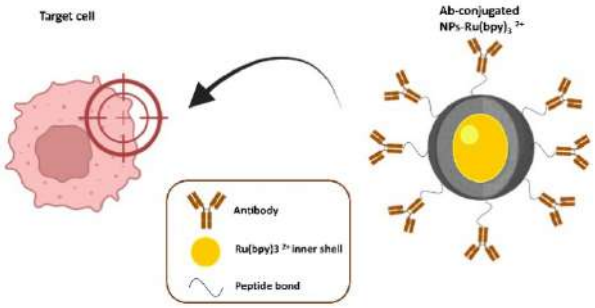

Figure. Structure of the antibody bioconjugated with $Ru(bpy)_3^{2+}$-doped silica nanoparticles and prostate cancer cells, our target.

Acknowledgements

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Unravelling the epigenetic mechanisms underlying the occupational carcinogenesis of hexavalent chromium

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Keywords: Epigenetics, biomonitoring, workers, biochemistry, prevention

Introduction. An estimated 900,000 workers across the European Union are occupationally exposed to hexavalent chromium (Cr(VI)), a known human carcinogen. Genotoxic effects, including oxidative stress and DNA damage, are well-established contributors to Cr(VI)-induced carcinogenesis. Emerging evidence also suggests that epigenetic mechanisms—particularly DNA methylation—may play a role in mediating the carcinogenic potential of Cr(VI). Objective. This study aimed to investigate epigenetic alterations associated with occupational exposure to Cr(VI). Methods. We conducted a cross-sectional study involving 254 workers from seven European countries with potential Cr(VI) exposure. Internal exposure was assessed by measuring urinary chromium levels. Biomarkers of biological effect—8-hydroxy-2'-deoxyguanosine (8-OHdG), global DNA methylation, and global DNA hydroxymethylation—were quantified in blood samples. A control group (n = 114) consisted of office workers either employed at the same companies as the exposed workers ("within-company controls") or from unrelated companies with no Cr(VI)-related activities ("outwith-company controls"). Results. All exposure subgroups had significantly higher mean urinary chromium concentrations than the total control group, the within-company controls, and the outwith-company controls ($p < 0.007$, Mann-Whitney test). Within-company controls also exhibited significantly higher internal exposure levels compared to outwith-company controls ($p < 0.001$). Moreover, outwith-company controls showed significantly higher levels of global DNA methylation and lower levels of 8-OHdG than all exposure subgroups ($p < 0.01$). Discussion and Conclusion. These findings underscore the value of internal exposure biomarkers and reveal that even office personnel in Cr-related industries may experience biologically relevant Cr exposure. The observed alterations in biomarkers of effect, particularly global DNA methylation, highlight the potential of epigenetic markers to improve occupational health risk assessment and management in environments involving Cr(VI).

Magnetite mineralization inside cross-linked protein crystals

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Keywords: Add a maximum of 5 keywords (size 9, Candara) Nanoparticles of magnetite, crystallisation in confinement, cross-linked protein crystals

Crystallisation in confined spaces is a widespread phenomenon in nature. Many processes like frost heave, biomineralization (bones and seashells), growth of salt and ice crystals in the pores of masonry happen not in a bulk, but in limited volumes. Reducing the volume of the system influences not only the probability of nucleation, but also the kinetic. As the results, in reduced volumes become possible stabilisation of metastable polymorphs, formation of crystals with preferred orientations, modification of morphology, etc. [1]
One of the most fascinating cases of crystallisation in confined spaces – formation of magnetite nanoparticles inside magnetotactic bacteria. The particles have unusual morphologies, homogeneous size and superparamagnetic properties. [2]
The control of the magnetite nanoparticles formation could be explained by a combination of physical and chemicals factors in which nucleation and growth of magnetite crystals is performed in confined vesicles (magnetosomes) modulated by the interaction with different proteins. [3]
In this work, we imitate this type of control over the precipitation of magnetite using channels of cross-linked protein crystals (CLPCs). CLPCs with different pore size and amino acid decoration were used to study their influence on the magnetite precipitation.
We obtained gradient distribution of nanoparticles with a narrow size distribution of around 2 nm independently of the channel diameter size of the CLPCs. But the pores size influenced the stabilisation of the Fe-rich phase. In case of lysozyme (the smallest pore size), the amorphous metastable intermediates of magnetite were stabilised, while in glucose isomerase crystals, the amorphous phase were recrystallized into the crystalline state of magnetite.
Our results demonstrate control in the size and stability of those Fe-rich nanoparticles and the potential use of the CLPCs as excellent scaffolds to study the crystallisation in confinement.

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