Il contributo dei giovani chimici in Campania

Società Chimica Italiana
Sezione Campania

19 Luglio 2024

Università degli Studi della Campania Luigi Vanvitelli

Dip. di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche (DISTABiF)

LIBRO DEGLI ABSTRACT

Una iniziativa di

Società Chimica Italiana
Sezione Campania

con il supporto di

Università degli Studi della Campania Luigi Vanvitelli

ORDINE REGIONALE DEI CHIMICI E DEI FISICI DELLA CAMPANIA

labozeta la sicurezza nel laboratorio

Levanchimica

PICCIN deltek SHIMADZU

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Raffaele Cucciniello
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Programma

08:30 – 09:30	Registrazione/Benvenuto
09:30 – 10:00	Saluti istituzionali: Prof. Antonio Fiorentino (Direttore DISTABIF, Università degli Studi della Campania Luigi Vanvitelli), Prof.ssa Concetta Giancola (Presidente SCI Campania), Prof. Gianluca Farinola (Presidente SCI), Dott.ssa Rossella Fasulo (Presidente OCF Campania)
10:00 – 10:30	Plenaria (Aula A2): Prof. Roberto Fattorusso (Università degli Studi della Campania Luigi Vanvitelli). *Chairperson*: Prof.ssa Concetta Giancola, Prof. Luigi Russo
Sessione I	
10:40 – 13:00	Sessione I.A (Aula A2). *Chairperson*: Dott.ssa Clementina Acconcia, Prof. Luigi Russo
10:40 – 13:00	Sessione I.B (Aula B2). *Chairperson*: Dott.ssa Fabiana Piscitelli, Prof. Vincenzo Russo
10:40 – 13:00	Sessione I.C (Aula A3). *Chairperson*: Prof.ssa Stefania Terracciano Prof. Raffaele Cucciniello
13:00 – 14:00	Foto di Gruppo e Light lunch
Sessione II	
14:00 – 16:00	Sessione II.A (Aula A2). *Chairperson:* Dott.ssa Giovanna Valentino, Prof. Stefano Cinti
14:00 – 16:00	Sessione II.B (Aula B2). *Chairperson:* Prof.ssa Stefania Terracciano, Prof. Vincenzo Russo
14:00 – 16:00	Sessione II.C (Aula A3). *Chairperson:* Dott.ssa Fabiana Piscitelli, Prof. Gianluca D'Abrosca
16:00 – 16:30	Coffee break
Sessione III	
16:30 – 18:30	Sessione III.A (Aula A2). *Chairperson:* Dott.ssa Maria Della Valle, Prof. Raffaele Cucciniello
16:30 – 18:30	Sessione III.B (Aula B2). *Chairperson:* Prof.ssa Stefania Terracciano, Prof. Luigi Russo
16:30 – 18:30	Sessione III.C (Aula A3). *Chairperson:* Dott.ssa Fabiana Piscitelli, Prof. Vincenzo Russo
18:30 – 18:45	Saluti conclusivi

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Programma scientifico dettagliato

SESSIONE I.A			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Antonella Ilenia Alfano	Modular Synthesis of Benzoylpyridines Exploiting a Reductive Arylation Strategy	10:40
2	Gaetano Caputo	Molecular docking and UV-Vis analysis of 3-O-Methylfunicone β-cyclodextrin complex for antiviral drug development mechanism	10:50
3	Simona De Vita	Pharmacophore-based Inverse Virtual Screening and its application in accelerating the target identification and repositioning of bioactive compounds	11:00
4	Emis Ingenito	Novel 1,3,4-Oxadiazoles as Potent PD-L1 Antagonists: A One-Pot Synthetic Approach for Enhanced Immune Checkpoint Inhibition	11:10
5	Alessandro Landi	Simulation of organic mixed ionic and electronic conductors with a combined classical and quantum mechanical model	11:20
6	Valerio Loianno	Unveiling the competitive diffusion of binary gas mixtures in polymers: the case of carbon dioxide and alkanes in nanoporous-crystalline polyphenylene oxide	11:30
7	Antonella Miglione	Development of a screen-printed electrode for the detection of Alkaline phosphatase as biomarker for cancer diagnosis and monitoring	11:40
8	Prisco Prete	Development and validation of an eco-compatible UV-Vis spectrophotometric method for the determination of Cu^{2+} in aqueous matrices	11:50
9	Federica Dragone	Marine Strategy Framework Directive: nutrient analysis in seawater samples	12:00
10	Valeria Romanucci	Targeting METTL3-14 degradation by PROTAC technology: design, synthesis and biological evaluation of new promising library of degraders	12:10
11	Francesco Ferdinando Summa	Derivation of Nuclear Magnetic Shielding and Magnetizability in Open-Shell Systems Throughout a Semi-Relativistic Approach	12:20

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SESSIONE I.B			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Susy Brusco	PEI-engineered lipid@PLGA hybrid nanoparticles for delivery of antigens and immune adjuvants through the respiratory mucosa	10:40
2	Carolina Cané	The antimicrobial peptide Esc(1-21)-1c increases susceptibility of Pseudomonas aeruginosa to conventional antibiotics by decreasing the expression of the MexAB-OprM efflux pump	10:50
3	Irene Contento	Gold nanoparticles supported on Poly(2,6-dimethyl-1,4-phenylene oxide) as catalyst: from oxidation processes to hydrogen production	11:00
4	Fulvio De Paola	Adsorption properties of a hybrid geopolymer toward Y^{3+} ions	11:10
5	Flavia Manuguerra	Marine Strategy Framework Directive: metal analysis in marine sediments	11:20
6	Giarita Ferraro	Binding of $[V_4O_{12}]^{4-}$ and Unprecedented $[V_{20}O_{54}(NO_3)]^{n-}$ to Lysozyme	11:30
7	Rosanna Paparo	Process intensification for removal of new pharmaceutical compounds from water	11:40
8	Alessio Occhicone	Designing carbon nanoparticles-diatomite hybrids for wastewater remediation	11:50
9	Salvatore Impemba	In_2O_3@TiO_2/Cu_2O for H_2 development by solar energy	12:00
10	Carla Serri	Silibinin-loaded amphiphilic nanoparticles: A promising drug delivery system for lung cancer therapy	12:10
11	Lucia Sessa	Experimental and Theoretical Insights into a Novel Lightfast Thiophene Azo Dye	12:20
12	Michele Emanuele Fortunato	Kinetic investigation of Silybin A/B chemo-enzymatic acetylation in a flow milli-reactor	12:30

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SESSIONE I.C			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Clementina Acconcia	Structural and dynamic insight into the MT1 activation mechanism	10:40
2	Ester Colarusso	Identification of 1-ethyl-1H-pyrazolo[3,4-b]pyridine-based compounds as new BRD9 binders	10:50
3	Maria della Valle	Understanding the PED/PEA15-Phospholipase D1 interaction mechanism in type II diabetes through NMR spectroscopy	11:00
4	Bianca Fiorillo	Structural Insights into the Agonist Mechanism of Action of Tetracyclic Antidepressants on Serotonin Receptors	11:10
5	Rosa Gaglione	Bioactive peptides hidden in human endopeptidases: the case of three novel cryptides identified in metalloproteinase 19 by a computational-experimental platform	11:20
6	Gabriella Pinto	Molecular fingerprint by omics-based approaches in saliva from patients affected by SARS-CoV-2 infection	11:30
7	Lucia Santorelli	Cross-linking Mass Spectrometry to decipher Cell Plasma Membrane Interactome	11:40
8	Gianluca D'Abrosca	Circular oligomers formed by Ros/MucR family members act as mediators of DNA condensation in α-proteobacteria	11:50
9	Claudia Finamore	Synthesis and interactome characterization of a novel cytotoxic quinazolinone library	12:00
10	Angela Di Somma	The antimicrobial peptide Temporin-L induces vesicle formation and reduces the virulence in S. aureus	12:10
11	Giovanna Valentino	NMR-based strategies for the rapid identification and characterization of anticancer specialized metabolites from Mediterranean Asteraceae species	12:20
12	Maria Carmina Scala	Rational design of novel peptidomimetics against influenza a virus biological and computational studies	12:30

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SESSIONE II.A			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Giovanna Aquino	Leaves for Life: Sustainable Green Extraction of Nutraceuticals from Agri-Food Waste	14:00
2	Francesco Taddeo	Ethyl levulinate ketalization with glycerol: from batch to continuous operation	14:10
3	Valentina Gargiulo	Possibilities and perspectives in the hybridization of metal organic frameworks (MOFs) with non-conventional graphene related material	14:20
4	Alessia Giannattasio	Polyethylene and polysiloxane persisting on surface seawater and in wastewater treatment plants: recovery, ponderal quantification, microstructural analyses and origin investigation	14:30
5	Alessandra Sessa	Comparative life cycle assessment of different synthetic routes of ZIF-8	14:40
6	Lorenzo Marino Cerrato	Development of a HPLC-MS/MS method for the characterization and quantification of intact glucosinolates in *Catozza* rapeseeds	14:50
7	Marco Morelli	Structural effects of the protein corona formed on different nanoparticles in the coelomic fluid of the sea urchin Paracentrotus lividus	15:00
8	Martina Pierri	Development of dual BRD9/HDAC hybrid ligands as novel epigenetic probes	15:10
9	Pasquale Rapacciuolo	Harnessing the isoxazole core to achieve hybrid LIFR-FXR modulation	15:20
10	Ciro Romano	Transition Metal-Catalysis for the Manipulation of Molecular Structure	15:30

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SESSIONE II.B			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Michele Costanzo	Multi-level proteomics defines multiple unbalances in methylmalonic acidemia	14:00
2	Simona Marzano	Unlocking the potential of protein-derived peptides to target G-quadruplex DNA: From recognition to anticancer activity	14:10
3	Elva Morretta	Functional proteomics-aided interactome analysis of bioactive pyrazolyl-ureas	14:20
4	Valeria Nele	mRNA-loaded Self-assembling Nanoparticles as Novel Vaccine Formulations	14:30
5	Maria Petrone	New Promising Curcumin Mimics as Neurodegenerative Hallmarks Rescuers	14:40
6	Vincenzo Piccolo	Optimization of Ursolic Acid Enriched-Oleolytes from Annurca Apple with Potential Depigmentation Activity	14:50
7	Mariantonietta Pizzella	Biomimicking amyloid aggregates for self-assembling biomaterials	15:00
8	Anna Illiano	Amino acid quantification by mass spectrometry without derivatization to characterize campanian cheeses	15:10
9	Verdiana Covelli	Probing FPPS enzyme inhibition: a multidisciplinary approach	15:20
10	Francesco Zaccaria	Quantitative analysis of self-diffusion coefficients (Dt): a semi-empirical model for aqueous solutions	15:30

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SESSIONE II.C			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Rosa De Gregorio	Drug repositioning a valid strategy for the identification of a new LIFR antagonist	14:00
2	Concetta Di Natale	Innovative ultra-sensitive detection of neurodegenerative biomarkers by the pyro-electrohydrodynamic jetting	14:10
3	Sara La Manna	Transition metal complexes versus amyloid aggregation: new potential therapeutic strategies in neurodegeneration	14:20
4	PanagiotaKalligosfyri	Paper-based assay utilizing sequence-specific dye for the colorimetric detection of TATA box	14:30
5	Andrea Rispo	Compatibilization of Isotactic Polypropylene (iPP) and Polyethylene (PE) with PP-based Block Copolymers	14:40
6	Fabrizio Villapiano	Thermosensitive in situ gelling poloxamers/hyaluronic acid gels for hydrocortisone ocular delivery	14:50
7	Ilaria Grimaldi	Stereoselective Polymerization of 1- Vinylcyclohexene and (S)-4-Isopropenyl-1-Vinyl-1-Cyclohexene and their Copolymerization with Styrene and Terpenes	15:00
8	Silvia Arino	Harnessing antimicrobial peptides in polymer membranes for water purification	15:10
9	Matilde Tancredi	Biosurfactant for eco-sustainable formulations: rhamnolipids as multifunctional component	15:20
10	Natalia Ventserova	Exploring Equilibria between Monomeric and Oligomeric species involved in Prion diseases	15:30

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SESSIONE III.A			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Giuseppe Femina	HNBR crystallization behavior under stretching: comparison between a semi-crystalline and an amorphous samples	16:30
2	Sara Vllahu	Highly efficient synthesis of zeolitic imidazolate framework-8	16:35
3	Antonietta Mollo	Innovative Oxoindoline Glutamine Mimic: Design and Synthesis of Peptidomimetic Inhibitors for SARS-CoV-2 3CLpro	16:40
4	Alessia Alberico	Design and Synthesis of a Novel Glutamine Mimetic Spiropyrrolidinone-Based P1 Moiety for M^{pro} Inhibitors	16:45
5	Ciro Migliaccio	Alkaline and acid Red mud-Metakaolin based geopolymers for adsorption of methylene blue	16:50
6	Emanuela Rizzo	Analysis of post-translational modifications (phosphorylation and N-Glycosylation) in proteins extracted from Tempera paintings	16:55
7	Wanda Cimmino	A printed electrochemical strip to evaluate nanovectors encapsulation	17:00
8	Anastasia Ferraro	A promising class of SARS-CoV-2 Mpro inhibitors: Spiropyrrolidinone-based compounds	17:05
9	Marica Chianese	Photodegradation kinetics of ibuprofen promoted by Fe-CeO2 catalysts active under visible light	17:10
10	Raffaele Marzocchi	Synthesis and characterization of β-myrcene/styrene and β-ocimene/styrene copolymers	17:15
11	Salvatore Mottola	Nucleobase functionalized peptides: a new strategy for targeting ATP and GTP in cancer cells	17:20
12	Angela Sorice	NMR-based profiling isolation and structural elucidation of potentially bioactive oleanane saponins from *Bellis sylvestris Cyr*.	17:25
13	Gennaro Battaglia	Mass spectrometry methods for the evaluation of saffron activity in retinal diseases	17:30
14	Annalisa De Cicco	Synthesis of new organocatalysts based on cyclic peptoids	17:35
15	Giuseppe De Marino	Ammonia Decomposition Using a Red Mud-Based Geopolymeric Catalyst for COx-Free Hydrogen Production	17:40
16	Francesca Fantasma	Phytochemical analysis of essential oil and methanolic extract from leaves of wild Origanum vulgare L. from central Italy, *in vitro* antioxidant activity and on HepG2 cell line	17:45
17	Fabiana Tescione	Engineered silica nanoparticles for wastewater remediation	17:50
18	Simone Davide	Compatibilization of isotactic polypropylene (iPP)/polyeathylene (PE) blends with iPP-graft-PE copolymers	17:55
19	Carlo Carandente Coscia	Innovative Formulations for Agricultural Applications	18:00
20	Angelo Santoro	New Aβ(1-42)ligands from anti-amyloid antibodies: Design, synthesis, and structural interaction	18:05

21	Ersilia Villano	Production of hybrid lipid/polymer nanoplatforms for RNA delivery by an emulsion-solvent diffusion technique: from benchtop to microfluidics	18:10
22	Emanuele Carrella	Metal-free synthesis of selenoglycosylated eumelanin monomers	18:15
23	Alessia Cugudda	Cyclic peptidomimetics as suppressor of cytokine signalling 1 (SOCS1)	18:20

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SESSIONE III.B			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Ilaria Neri	Alternative tools for rapid and high throughput assessment of transdermal passage	16:30
2	Miriam Di Martino	Cationic Azobenzenes as Light-Responsive Crosslinkers for Alginate-Based Supramolecular Hydrogels	16:35
3	Nicola Grasso	Exploring the interaction between DNA G-quadruplexes and an RG-rich peptide	16:40
4	Claudia González Castro	Mimics of glutathioneperoxidase: selenoglycoconjugates	16:45
5	Francesco Viceconte	Novel sulfonated n-heterocyclic carbene silver(I) and gold(I) complexes in a^3-coupling catalysis	16:50
6	Angelo Fenti	Continuous-flow electrochemical oxidation of nitrogen compounds in livestock manure with an innovative reactor: The RiduciN Project	16:55
7	Danila La Gioia	Enhancing Sensitivity and Robustness in Untargeted Metabolomics:Microbore UHPLC-HRMS Approach	17:00
8	Paolo Scognamiglio	Valorization and Metabolomic Analysis of Trub as a Sustainable Resource for the Pharmaceutical and Food Sector: A Circular Approach	17:05
9	Andrea Criscuolo	Dimer optimization of G-quadruplex-forming aptamers	17:10
10	Claudio Clemente	Metal–Organic Framework-derived ZnO with Enhanced Ethanol Sensing Properties	17:15
11	Luciana Cimino	Invasive seaweeds and their hybrids as natural sorbent for wastewater remediation	17:20
12	Matteo Delli Carri	Metabolic Profiling of Plasma Distinguishes Indolent from High-Grade IPMNs using GC-MS	17:25
13	Gilda D'Urso	Metabolomic Profiling of Fecal Samples from Breastfed Late Preterm Infants Compared to Those Fed Postbiotic-Supplemented Formula Milk	17:30
14	Dafne Ruggiero	A Multidisciplinary Strategy for the Identification of a Novel Thiadiazolopyrimidone Targeting Annexin A6	17:35
15	Lucio Spinelli	Optimization of a Nanoparticles Protein Corona Isolation and Identification Platform using Omics	17:40
16	Emmanuel De Gregorio	Polybenzimidazole-based electrospun membranes for fuel cell application	17:45
17	Alessandro Salvati	GC analysis and microbiological analysis of lemon essential oils stored for 25 years	17:50
18	NiloufarKeivani	Optimized Extraction of Phenolics and Procyanidins from Seven Medicinal Herbs for Nutraceutical Development	17:55
19	Giuseppe Caso	Particulate matter source apportioning by means of Pb stable isotope ratio measurements	18:00

20	Alessandra Capuano	Target Discovery of Natural Myrianthic Acid Through Label-Free Proteomics and Mass Spectrometry Approach	18:05
21	Maddalena Paolillo	Non-covalent Interactions with Proteins of Potential Vanadium Drugs: The Case of the Lysozyme/VIVO-8-hydroxyquinoline adduct	18:10
22	Michela De Luca	Methylene blue adsorption from aqueous matrix on geopolymer-based substrates	18:15
23	Armando Astone	Catalytic Asymmetric Approach to 1,3,4,5-Tetrahydro-1,4-benzodiazepin-2-ones in One-Pot	18:20

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SESSIONE III.C			
N	**Oratore**	**Titolo Presentazione**	**Orario**
1	Carolina Fontanarosa	POPs determination in serum and semen of contaminated areas of northern Italy	16:30
2	Michele Spinelli	Lanthionine determination in serum of patients affected by Chronic Kidney Disease by Multiple Reaction Monitoring	16:35
3	Vadym Samukha	Metabolomic Analysis of Malus domestica (Suckow) Borkh. Varieties From Molise Region (Italy) by NMR Spectroscopy	16:40
4	Francesca Scala	Targeting c-Myc: Discovery and Enhancement of New Diphenyl Urea-Based Inhibitors for Cancer Treatment	16:45
5	Carla Aliberti	Improving the Biological Properties of Thrombin-Binding Aptamer by Incorporation of 8-Bromo-2'-Deoxyguanosine and 2'-Substituted RNA Analogues	16:50
6	Martina Ridinò	Bile acid derivatives as LIFR/LIF inhibitors	16:55
7	Anna Guadagni	Discovery Of A New Potent And Selective Histone Deacetylase 6 Inhibitor In Triple Negative Breast Cancer Treatment	17:00
8	Daniela Benigno	G-Quadruplex Aptamers as Promising Inhibitors of the STAT3 Signaling Pathway	17:05
9	Maryam Kamarehei	Development of peptide-based molecular strategies to interfere with protein misfolding and aggregation of prion	17:10
10	Rosanna Lucignano	Globular proteins as suitable tool to obtain amyloid fibrils	17:15
11	Michela Buonocore	Investigation of the disassembly/reassembly mechanism of a human-derived recombinant ferritin	17:20
12	Martina De Rosa	Identification of Ligands targeting Gs protein by Ligand-based NMR spectroscopy	17:25
13	Brunella Cipolletta	Binder-pigment interaction by proteomic approaches	17:30
14	Mattia Cammarota	Natural products from poly-extremophilic marine fungi in the treatment of emerging infectious diseases	17:35
15	Erica Gazzillo	Identification of new c-Myc binders through a combined in silico and STD/NMR-based approach	17:40
16	Michela Aliberti	Virtual screening of 3-(1H-1,2,3-triazol-1-yl)piperidine-2,6-dione-based compounds as new potential Cereblon modulators amenable for targeted protein degradation	17:45
17	Carlo Raucci	2D-NMR in structural elucidation of specialized metabolites from Trichoderma spp. bioactive fractions	17:50
18	Stefania Serpico	A rapid liquid chromatography/mass spectrometry method to identify promising protein biomarker of COVID-19 infection	17:55
19	Flavia Conte	Validation of analytical methods for trace elements determination in biological matrices	18:00
20	Enza Napolitano	Analyzing nicotine protection mechanism against amyloid toxicity by NMR-metabolomics: an exploratory study	18:05

21	Simone Braccia	Peptide-based nanofibers for a selective drug delivery in glioblastoma treatment	18:10
22	Antonella Vitiello	HPβCD Impact on Zein Edible Films for Food Packaging	18:15

Structural and dynamic insight into the MT1 activation mechanism

Clementina Acconcia[1*], Antonella Paladino [2], Maryam Kamarehei[1], Francesca Scebba[3], Debora Angeloni[3,4], Stefano Comai[5], Luigi Russo[1]

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5 - Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy.

Melatonin, mainly acting through its two receptors MT1 and MT2, plays a crucial role in regulating circadian rhythms and in other physiological processes [1-4]. Melatonin receptors seem to exhibit distinct roles in rapid eye movement (REM) sleep compared to non-rapid eye movement (NREM) sleep [5]. Various preclinical studies utilizing knockout animals for MT1 and MT2 receptors suggest that these melatonin receptors may possess complementary physiological functions [4]. However, the pathophysiological function of the MT1 and MT2 receptors is still poorly understood. Here, in order to enhance our comprehension on the MT1 recognition mechanism by ligands, we conducted structural investigations into MT1 binding by UCM871 and Melatonin utilizing a multidisciplinary approach including Nuclear Magnetic Resonance (NMR), Molecular Modeling and Molecular Dynamics and Docking methodologies. Initially, to delineate the MT1 binding sites of ligands, we conducted saturation transfer difference (STD) and T1ρ experiments in the presence and in absence of cell membranes containing the MT1 receptor. Subsequently, we built a three-dimensional model of the active MT1 in complex with ligands by integrating computational methodologies with NMR data. Overall, our findings illustrate that the MT1 activation process, involving structural rearrangements of the cytoplasmic side of the receptor, is mainly driven by hydrophobic interactions of both ligands with residues surrounding the orthosteric site of the receptor.

Keywords: *solution NMR, molecular modelling, membrane receptor, biomolecules.*

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References

[1] M. L. Dubocovich, M. Markowska, Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 27, 101-110 (2005).
[2]. Comai S, Lopez-Canul M, De Gregorio D, Posner A, Ettaoussi M, Guarnieri FC, Gobbi G. Melatonin MT1 receptor as a novel target in neuropsychopharmacology: MT1 ligands, pathophysiological and therapeutic implications, and perspectives. Pharmacol Res. 2019 Jun;144:343-356.
[3]. B. Lacoste, D. Angeloni, S. Dominguez-Lopez, S. Calderoni, A. Mauro, F. Fraschini, L. Descarries, G. Gobbi, Anatomical and cellular localization of melatonin MT1 and MT2 receptors in the adult rat brain. J. Pineal Res. 58, 397-417 (2015).
[4]. Gobbi G, Comai S. Differential Function of Melatonin MT1 and MT2 Receptors in REM and NREM Sleep. Front Endocrinol (Lausanne). 2019 Mar 1;10:87.
[5] Gobbi G, Comai S, Differential Function of Melatonin MT. Front Endocrinol (Lausanne) (2019) 10:87.

Design and Synthesis of a Novel Glutamine Mimetic Spiropyrrolidinone-Based P1 Moiety for M^{pro} Inhibitors

Alessia Alberico [1*], Anastasia Ferraro[1], Antonietta Mollo[1], Rolando Cannalire[1], Vincenzo Summa[1]

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Since the onset of the COVID-19 pandemic, the main protease (M^{pro}) of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has emerged as a primary target for the development of novel direct antiviral agents (DAAs)[1]. It is a cysteine protease responsible for the polyproteins cleavage pp1a and pp1ab in non-structural proteins (NSPs), essentials for viral replication. It recognizes a unique cleavage site featuring a glutamine residue at the P1 position, which is not utilized by human proteases. Moreover, the highly conserved catalytic site within the Orthocoronavirinae subfamily underscores the potential for developing Pan-CoV inhibitors.

Here, we outline the synthesis of a novel P1 moiety based on a (3R, 5'S) - spiropyrrolidinone structure, as a cyclic glutamine mimetic. The new P1 has been designed to replace the well-known γ-lactam ring found in the first-in-class inhibitor of SARS-CoV-2 M^{pro}, Nirmatrelvir[2]. The pyrrolidinone of the spirocyclic moiety in the P1 position can retain the key hydrogen bonds between the amide group with the side chains of His163 and Glu166 residues; in addition, the aromatic ring of spiropyrrolidinone can make further π-π stacking interactions with the backbone of Phe140 within the S1 pocket of the binding site. These interactions play a pivotal role in enhancing affinity, potency, and specificity towards this cysteine protease.

The synthesis was performed in a multigram scale in five reaction steps, starting from the esterification of L-tryptophan, following the Pictet-Spengler reaction to afford a condensed tricyclic intermediate. After the protection of the amine with the tert-butoxycarbonyl group, the so-called pinacolic rearrangement takes place in the presence of N-Bromo Succinimide and Acetic Acid, to obtain the spiropyrrolidinone core. The two diastereoisomers were separated, and the pure enantiomer (98% ee) underwent the removal of the Boc protecting group with HCl 4N dioxane solution to achieve the hydrochloride salt as the final compound, with an overall yield of 80%.

The (3R, 5'S) spiropyrrolidinone P1 moiety can be considered a new isoster of the P1 γ-lactam ring, able to fulfill the S1 subpocket of SARS-CoV-2 M^{pro}, providing the basis for the design and synthesis of a novel class of peptidomimetic inhibitors.

Keywords: *SARS-CoV-2, M^{pro} Inhibitors, Spiropyrrolidinone moiety, New Chemical Entities (NCEs)*

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References

[1] Cannalire R., Summa V., J. Med. Chem. (2022) 65, 4, 2716–2746
[2] D.R. Owen et al. Science (2021) 374(6575):1586-1593

Modular Synthesis of Benzoylpyridines Exploiting a Reductive Arylation Strategy

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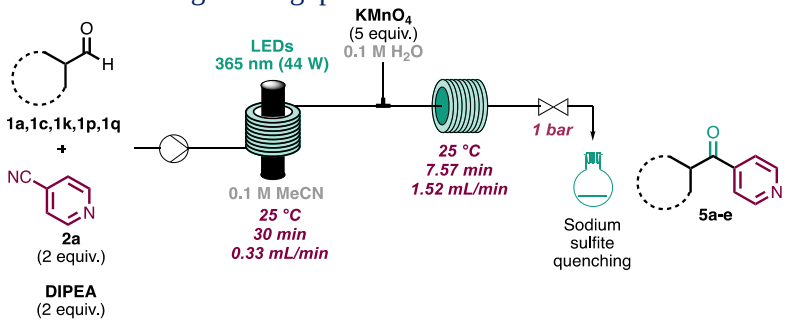
Developments in modern photocatalysis have highlighted the power of Ru- and Ir-based catalysts to bring about a plethora of valuable synthetic processes using visible light.[1] Due to the high cost and potential toxicity, their use in industrial settings is fairly limited. In this context, simple organic molecules with sufficient conjugation continue to play a key role in scaled photochemical reactions and triplet photosensitizers such benzophenone and (thio)xanthone are frequently used examples. While these entities are readily available at low cost, display good solubility and are considered non-harmful, the introduction of electron-donating or -withdrawing substituents that is critical to modify their photophysical properties, commonly necessitates long and inefficient synthesis routes. To address this challenge, we set out to create an expedited route into electronically differentiated bis-aryl ketones that combine electron-rich benzene systems with electron-deficient pyridyl moieties. Continuous flow processing was employed to provide increased scalability, reaction efficiency as well as reproducibility.[2] Our approach merges a light-driven (365 nm) and catalyst-free reductive arylation between aromatic aldehydes and cyano-pyridines with a subsequent oxidation process with $KMnO_4$.[3] The addition of electron donating and -withdrawing substituents on the scaffold allowed to effectively modify the absorbance of these compounds in the UV-Vis region while the continuous flow process affords high yields, short residence time and high throughput.



Keywords: *flow chemistry, photochemistry, catalyst-free, photosensitizers*

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Improving the Biological Properties of Thrombin-Binding Aptamer by Incorporation of 8-Bromo-2'-Deoxyguanosine and 2'-Substituted RNA Analogues

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Thrombin binding aptamer (TBA) is one of the best-known G-quadruplex (G4)-forming aptamers that efficiently binding to thrombin resulting in anticoagulant effect [1]. The major limit to TBA therapeutic application is represented by its poor thermal and biological resistance. Therefore, numerous research studies have been focused on the design of TBA analogues with chemical modifications to improve its pharmacokinetic and pharmacodynamic properties. Preserving the canonical antiparallel topology of the TBA quadruplex core is necessary to maintain the functional recognition to protein surface on which TBA anticoagulant activity depends [2]. To evaluate the effects of nucleobase and sugar moiety chemical modifications on biological properties of TBA, we have designed three TBA variants with modified G-tetrads preserving its chair-like G-quadruplex structure. All derivatives contain 8-bromo-2'-deoxyguanosine (GBr) in positions adopting a *syn* glycosidic conformation, while in positions adopting an *anti* conformation, locked nucleic acid guanosine, 2'-O-methylguanosine and 2'-F-riboguanosine have been introduced in TBA analogues TBABL, TBABM and TBABF, respectively.
CD (Circular Dichroism), CD melting, ^{1}H-NMR (Nuclear Magnetic Resonance), and non-denaturing PAGE (Polyacrylamide Gel Electrophoresis), nuclease stability, prothrombin time (PT) and fibrinogen-clotting assays have been performed to investigate the structural and biological properties of these TBA analogues. The most interesting results have been obtained with TBABF, which revealed: 1) extraordinary thermal stability (T_m approximately 40°C higher than that of natural TBA), 2) an anticoagulant activity almost doubled compared to the original aptamer and 3) a never-observed resistance to nucleases (50% of its G4 species was still present in 50% FBS at 24 h [3]). These data indicate TBABF as one of the best TBA analogue ever designed and investigated, to the best of our knowledge, overcoming the main limitations to therapeutic applications of this aptamer.

Keywords: 8-bromo-2′-deoxyguanosine; G-quadruplex; RNA analogues; anticoagulant activity; thrombin binding aptamer.

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Virtual screening of 3-(1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione-based compounds as new potential Cereblon modulators amenable for targeted protein degradation

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Cereblon (CRBN) is a ubiquitin ligase E3 substrate receptor protein that facilitates the transfer of ubiquitin to a protein of interest (POI) through reversible interactions with the CRL4 core complex, which consists of the Cullin-4 scaffold, the DNA damage-binding protein 1 (DDB1) adaptor protein, and RING finger protein ROC1[1]. Due to its pivotal role in protein ubiquitination, CRBN is commonly targeted for triggering the degradation of a POI. Several modulators (e.g., thalidomide, pomalidomide, lenalidomide) were identified as able to enhance the transition from an open to a closed conformation of CRBN and they act as molecular glues, thereby facilitating the recruitment and subsequent degradation of the POIs (e.g., IKZF1, IKZF2, CK1α, GSPT1)[1] . Also, they are used as E3 ligands in the design of novel Proteolysis Targeting Chimeras (PROTACs) heterobifunctional molecules. However, CRBN modulators are often unstable and easily undergo hydrolysis in body fluids[2] and, accordingly, the identification of novel molecules is urgently needed.
To identify novel CRBN binders with improved chemical stability, we generated a virtual library of 3-(1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione-based compounds: 1838 commercially available terminal alkynes were combined with 3-azido-2,6-piperidinedione for producing a final set of 1838 compounds (CombiGlide software[3]), whose pharmacokinetic properties were subsequently predicted (QikProp software[4]). The filtered library with a favorable ADME profile was used as input for a molecular docking-based virtual screening campaign on CRBN. The analysis of docking scores and interactions with CRBN key amino acids led to the selection of 12 new items for the next stages of chemical synthesis and biophysical/biological evaluation, which is ongoing. In the future, we plan to replace the E3 ligase molecular portion of known PROTACs with the new identified CRBN modulators, with the final aim of improving pharmacokinetic properties and degradation efficiency of these heterobifunctional molecules.

Keywords: *Cereblon, molecular glue, targeted protein degradation*

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Leaves for Life: Sustainable Green Extraction of Nutraceuticals from Agri-Food Waste

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This project aimed to valorize agricultural by-products by investigating different leaves' species deriving from *Allium cepa*, *Solanum lycopersicum* and *Olea europaea*, with the purpose of exploit and allow "a second life" to these precious by-products and to obtain high-value added extracts suitable for various applications in the food and pharmaceutical industries. *Allium cepa* PDO (Protected Designation of Origin) leaves hydroalcoholic extract was obtained by a Box Behnken Design – Microwave Assisted Extraction model previously optimized [1]. To provide a better separation and to resolve the complete chemical profile of the extract, advanced analytical technique like comprehensive two-dimensional (2D) liquid chromatography (LC × LC) was developed. Olive leaves extract from Olevano sul Tusciano, due to the high sugar content, was subjected to a Solid Phase Extraction for the purification of the polyphenolic components. Phytochemical composition of two tomato PGI (Protected Geographical Indication) varieties leaves was investigated and to improve the solubility and stability of identified alkaloids (α-tomatine, α-TM and tomatidine, TD), nanoformulations α-TM-SLN and TD-SLN were prepared and characterized. Leaves extracts were characterized by ESI-Orbitrap-MS/MS platform and their potential activities were evaluated in different cell lines. In detail, optimal onion extract metal binding capacity and the ability to inhibit the intracellular reactive oxygen species (ROS) release on hepatocarcinoma cell line (HepG2) using an H_2O_2-induced oxidative stress model, were evaluated. HepG2 cells were also treated with a mixture of free fatty acids (oleic acid: palmitic acid, 2:1) to simulate non-alcoholic fatty liver disease (NAFLD) and to study the possibility of olive leaves extract to prevent NAFLD. Finally, potential anticancer activity of both tomato PGI extracts was evaluated *in vitro* to assess the effect of α-TM and TD on the percentage of cellular viability in Olfactory Ensheathing Cells (OECs) and in SH-SY5Y, a neuroblastoma cancer cell line. Based on our results, leaves extracts could be regarded as a valuable source of bioactive compounds, suitable for various applications in nutraceutical and onconutraceutical fields.

Keywords: *agri-food waste, leaves, nutraceutical, green extraction*

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Harnessing antimicrobial peptides in polymer membranes for water purification

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The lack of clean drinking water is a serious problem worldwide, especially in developing countries. Thus, an hot research topic deals with the development of practical and sustainable systems to supply communities in need with clean drinking water. Antimicrobial peptides (AMPs) show promise in this regard because of their full biodegradability and exceptional efficacy against target infections. AMPs are typically characterized by their short size, strong cationic nature, and propensity to assume amphipathic structures. Owing to their properties, these peptides can bind to negatively charged bacterial membranes and effectively kill pathogens through different mechanisms of action [1]. In this context, our research is aiming to create water purification filters using de novo antimicrobial peptides immobilized on polymer supports. In particular, starting from reported antimicrobial sequences [2], we have applied machine learning algorithms [3] for discovering new potential AMPs with enhanced activity.
After synthesis and characterization of the selected sequences, peptides have been implemented in a polymeric matrix by a "grafting through" strategy. The resulting compounds have been thoroughly characterized by differential scanning calorimetry (DSC), X-ray diffraction, UV-vis, and NMR spectroscopy. Further, the antimicrobial properties of the selected AMPs have been investigated both in their free and polymer-bound forms, showing that they maintain bactericidal activity after immobilization. Overall, these results highlight the viability of our approach for the development of water filtering membranes.

Keywords: *antimicrobial peptides, AMPlify, functionalized membranes*

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Catalytic Asymmetric Approach to 1,3,4,5-Tetrahydro-1,4-benzodiazepin-2-ones in One-Pot

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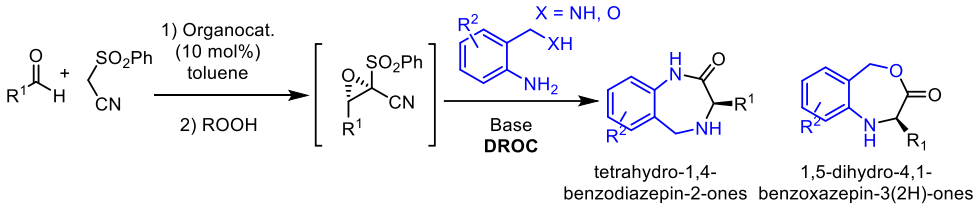
Tetrahydro-1,4-benzodiazepin-2-ones are significant scaffolds in medicinal chemistry, endowed with a variety of bioactivities, including applications in CNS treatment. Generally, they contain one or more stereocenters and the classical asymmetric approach relies on the use of enantiopure starting materials derived from the chiral pool.[1]
So far, the methods reported suffer from several limitations: in many cases, they are non-catalytic processes and often the possibility of introducing functional groups at the chiral center is limited. Given this background, the development of catalytic stereoselective methodologies for the synthesis of functionalized enantioenriched tetrahydro-1,4-benzodiazepin-2-ones is highly desirable.
Recently, our research group demonstrated the utility of enantioenriched 1-cyano-1-phenylsulfonyl epoxides as key intermediates in developing organocatalytic one-pot asymmetric processes aimed at synthesizing medicinally relevant compounds such as dihydroquinoxalinones, piperazin-2-ones, morpholin-2-ones, α-aryl glycine and aliphatic α-amino acid methyl esters.[2] The synthetic protocols consist in Knoevenagel reaction/asymmetric epoxidation/domino ring-opening cyclization (DROC) sequence, where the first two steps are promoted by a recyclable organocatalyst derived from Cinchona alkaloids. A similar strategy has been devised to accomplish the asymmetric synthesis of 1,4-dihydro-4,1-benzoxazepin-3(2H)-ones starting from readily available commercial reagents. This route gives access to a novel class of seven-membered N- and O-heterocycles, differently functionalized at the chiral center.[3]



Keywords: *asymmetric catalysis, epoxides, one-pot reaction, seven-membered heterocycles.*

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Mass spectrometry methods for the evaluation of saffron activity in retinal diseases

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Saffron is a highly prized spice frequently utilized as an additive due to its color-enhancing and flavoring properties, as well as its medicinal potential. Its intricate molecular composition has become the central focus of interest because of its several beneficial interactions across diverse pathological conditions [1,2].The anti-apoptotic properties of saffron's principal constituents [4] , along with its role in oxygen diffusibility [5] , make it particularly useful in the context of retinal pathologies. Over the last decade, extensive efforts have been undertaken to elucidate the potential of this precious spice. Presently, various therapeutic attributes have been ascribed to it, including a potential neuroprotective role in neurodegenerative retinal pathologies [3]. During this research at Federico II of Naples in collaboration with Hortus Novus srl, metabolomics and proteomics study were conducted. Animal model studies (murine) were conducted, leveraging the instrumental sensitivity of mass spectrometry in Multiple Reaction Monitoring (MRM) mode to identify molecules that traverse the emato-retinal membrane (ERM). We focused on MRM crocins panel associated to spice often used to characterize the saffron. Proteomic studies were initiated by analyzing tear samples from patients involved in a trial program with a saffron-based supplement. The main goal is to be able to identify possible markers from the tear matrix to monitor patient health status and further elucidate mechanisms already known in the literature involved in neurodegeneration. Several retina-related neurodegenerative diseases are doing under study. We focused on samples with age-related macular degeneration (AMD). With the support of Hortus Novus to date we are monitoring the regulation over time of a specific panel of proteins identified by mass spectrometry that appear to be up- and down-regulated compared to healthy patients.

Keywords: *Mass spectrometry, High-Performance Liquid Chromatography, saffron, retinal pathologies, characterization.*

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G-Quadruplex Aptamers as Promising Inhibitors of the STAT3 Signaling Pathway

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DNA and RNA aptamers are relatively short synthetic nucleic acids able to adopt distinctive three-dimensional structures and bind, with high affinity and specificity, a wide variety of molecular targets [1]. Many aptamers, with significant biological activities, are characterized by G-rich sequences, thus adopting G-quadruplex structures (G4s) as scaffolds [2]. The outstanding polymorphism and stability of the G4s are the key features that make the G4-aptamers one of the most investigated compounds in the field of therapeutics. T40214 (**STAT**) [$(G_3C)_4$] is a G4 aptamer that can efficiently influence STAT3 biological outcomes in several cancer cells [3]. Targeting the STAT3 protein through high-affinity ligands to reduce its levels or activity in cancer has noteworthy therapeutic potential.
To identify a new aptamer with antiproliferative activity and to evaluate the role of an extra cytidine in the second position of the T40214 sequence, the G4 forming oligonucleotide, namely **STATB** [$GCG_2(CG_3)_3C$], has been designed. Furthermore, to explore the effects of single site-specific replacements of loop residues in generating aptamers that can affect the STAT3 biochemical pathway, a series of **STAT** and **STATB** analogues containing a thymidine residue instead of cytidines was prepared. NMR, CD, UV, and PAGE data suggested that all derivatives adopt dimeric G4 structures like that of unmodified T40214 endowed with higher thermal stability, keeping the resistance in biological environments substantially unchanged, as shown by the nuclease stability assay. The antiproliferative activity of these ODNs was tested on both human prostate (DU145) and breast (MDA-MB-231) cancer cells. All derivatives showed similar antiproliferative activities on both cell lines, revealing a marked inhibition of proliferation, particularly at 72 h at 30 μM. The transcriptomic analysis, aimed to evaluate **STAT**'s and **STATB**'s influence on the expression of many genes in MDA-MB-231 cells, suggested their potential involvement in STAT3 pathway modulation. These data provide new tools to affect an interesting biochemical pathway and to develop novel anticancer and anti-inflammatory drugs.

Keywords: G-quadruplex, aptamers, antiproliferation, STAT3

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Peptide-based nanofibers for a selective drug delivery in glioblastoma treatment

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Glioblastoma (GBM) is one of the most aggressive malignant brain tumors and it is characterized by poorly differentiated astrocytes. GBM is associated to dysregulation of intracellular signaling pathways that involving protein p53 and the receptor tyrosine kinase. The conventional therapies are based on surgery, chemotherapy, and radiotherapy[1]. One of the limits of chemotherapy are the pharmacokinetics properties of drugs and the ability to cross the blood-brain barrier (BBB). Herein, with the aim to overcome these drug's limitations, we designed and developed a self-assembled peptide-based nanofiber finalized to a selective on-demand drug release into GBM cells[2]. The nanofiber is composed of two structural self-assembled peptides that contain negative and positive charged amino acids featured by the presence of an aliphatic sequence of six alanine residues and a lipid tail (nonadecanoic acid) linked to the amino group of lysine in C-terminal[3]. On the external surface, the nano-system is decorated by i) the drug Temozolomide (TMZ), a lipophilic chemotherapeutic, ii) the cell-penetrating peptide (CPP), namely gH625 that promotes cellular internalization, and iii) the targeting peptide. Chemotherapeutic drug TMZ is conjugated to a cleavage sequence recognized by the matrix metalloproteinase-9 (MMP-9), which is over-expressed in GBM cells. All peptides were obtained by solid-phase peptide synthesis (SPPS); dynamic light scattering, zetametry, and fluorescence assays, were performed for determining the size, charge, and critical aggregation concentration (CAC) of nanofibers, and their formation was confirmed by transmission electron microscopy; finally biological assays were performed.

Keywords: *glioblastoma, peptide-based nanofiber, on-demand strategy*

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PEI-engineered lipid@PLGA hybrid nanoparticles for delivery of antigens and immune adjuvants through the respiratory mucosa

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Delivering vaccines through the mucosal surfaces of the respiratory tract represents an intriguing, needle-free alternative to parenteral administration. Additionally, the mucosal immune system is the largest lymphoid tissue in the human body, capable of eliciting a robust local or systemic immune response and activating B- and T-cells. To effectively harness these benefits for both preventive and therapeutic immunization, a specially designed formulation is required [1; 2]. To this purpose, the use of nanovaccines, which exploit nanotechnologies to overcome biological barriers, is gaining growing attention. Along these lines, the aim of this study was the design and the development of a multimodal inhalable nanovaccine comprising a poly(lactic-co-glycolic) acid (PLGA) core, containing (Ova) as model antigen and a CpG motif as adjuvant. Given the key role that NP surface plays in engaging with the biological environment, we explored the effect of different moieties as surface coating. We tested i) 1,2 distearoil-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG), to achieve mucus-penetrating hNPs; ii) MPLA, to obtain both a mucoinert and responsive hybrid lipid/polymer nanoplatform. Finally, the addition of polyethyleneimine (PEI), to improve the efficiency of conventional vaccines was evaluated. A panel of Ova/CpG-loaded hNPs was produced and fully characterized in terms of size (i.e., hydrodynamic diameter or DH), polydispersity index (PDI) and ζ-potential. The hNPs architecture was evaluated through a combination of analytical tests, allowing to verify the presence and to quantify the specific moiety (i.e., PEG, MPLA or PEI) on the surface. The results collected where then combined with DSC and information related to the spatial distribution of the components and the internal structure of the optimized nanoplatforms. The impact of hNP features on their interactions with mucin and mucus penetration was investigated. Finally, selected formulations were tested for: i) uptake studies on murine dendritic cells (DCs), ii) antigen presentation tests in B3Z OT-I hybridoma cells expressing a TCR that specifically recognize OVA; iii) pro-inflammatory potential on human monocytes. Preliminary results highlight the potential of the developed nanoplatforms to successfully deliver antigens to the respiratory mucosa.

Keywords: *Mucosal delivery, Vaccination, Immunization, Nanoparticle, Antigen*

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Investigation of the disassembly/reassembly mechanism of a human-derived recombinant ferritin

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Ferritins are natural ubiquitarian proteins which spontaneously self-assemble adopting quaternary structures composed of 12 or 24 subunits. This event causes the formation of ordered nanocages with a hollow core able to store small and medium-sized molecules.[1] Thanks to this peculiar behaviour, there is a significant interest in ferritins in the scientific community, i.e. in using them as carriers in drug delivery or storage and in optimizing the conditions to enhance the yield of encapsulation in different environments.[2,3] Here we investigate the mechanism of disassembly and reassembly of a human-derived recombinant ferritin – constituted only by the heavy chain – (hHFt) exploiting a new protocol which involves the use of minimal amounts of the surfactant sodium dodecyl sulfate (SDS). The effectiveness of the protocol was analyzed in comparison with two commonly used methodologies based on the pH shift at acidic (pH 2) and alkaline (pH 13) conditions. This investigation was carried out using different techniques, like NMR and TEM, thanks to which it was possible to assume that small concentrations of SDS partially loosen the hHFt nanocage without detaching the monomers from each other, rather than totally disrupting the super-assembled system. Consequently, this mechanism resulted in a remarkable improvement in the protein recovery after the reassembly with respect to the pH shift; additionally, we verified that the partial opening allows the successful encapsulation of a small metallic complex in the protein with a high yield.

Keywords: *ferritin, drug delivery, disassembly protocol, NMR, TEM*

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Natural products from poly-extremophilic marine fungi in the treatment of emerging infectious diseases

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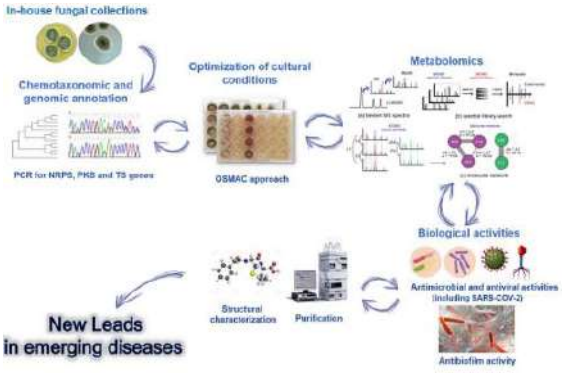
The unique features of marine environment, such as high salinity, hydrostatic pressure, low temperature and concentrations of organic matter, allows the development of poly-extremophilic organisms showing even more exciting abilities.[1] This is especially true for marine microbes representing a highly significant area for searching natural products, including novel therapeutic compounds with antimicrobial, anticancer and antiparasitic properties.
Marine fungi are a diversified source of bioactive metabolites that could be potentially used as new drugs. Their chemical and biological diversity is still underestimated, in fact, many new and neglected species need to be carefully investigated to search new bioactive metabolites.
In this presentation we showed the first results of our research. Several strains, available in Fenice's group, were selected and subjected in our laboratory to a standardized repartition procedure. The obtained extracts were tested for their antiviral, antimicrobial and antibiofilm activity and were analysed by mass spectroscopy to get a metabolic network.
The best candidates selected in this first stage will be cultivated according to the OSMAC[2] strategy, to stimulate the expression of silent secondary metabolite genes. For the best promising strains, production of SMs will be upscaled to obtain sufficient material for further activity tests and for the isolation, purification, and characterization of bioactive compounds.



Keywords: *marine environment, new compounds, emerging diseases.*
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The antimicrobial peptide Esc(1-21)-1c increases susceptibility of *Pseudomonas aeruginosa* to conventional antibiotics by decreasing the expression of the MexAB-OprM efflux pump

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The rise in bacterial strains resistant to conventional antibiotics represents a critical threat to human health, leading to future pandemics. Among the high number of bacterial pathogens implicated in severe infections, *Pseudomonas aeruginosa* (*P. aeruginosa*) stands out due to its remarkable resistance mechanisms and association with hospital-acquired infections. This scenario underscores the urgent need for novel antimicrobial agents or adjuvants capable of enhancing the efficacy of existing antibiotics. Recently, significant attention has been directed toward a frog-skin derived AMP, specifically Esc(1-21)-1c, a derivative of esculentin-1a. This peptide has exhibited potent antipseudomonal activity without cytotoxic effects on human cells, making it a promising candidate for further investigation [1]. The present study investigates the combined effect of Esc(1-21)-1c with various antibiotics, revealing that Esc(1-21)-1c synergistically inhibits the growth of *P. aeruginosa* when combined with three different antibiotics, including tetracycline. The underlying mechanism of action was explored using a differential proteomic approach, which showed a significant reduction in the production of three proteins associated with the MexAB-OprM efflux pump following treatment with sub-inhibitory concentrations of Esc(1-21)-1c. This down-regulation was confirmed through transcriptional analysis and direct quantification via tandem mass spectrometry in multiple reaction monitoring mode [2]. Consequently, the intracellular concentration of the antibiotic increases, rendering the bacteria more susceptible. In conclusion, the study underscores the critical importance of discovering and characterizing new molecules capable of synergizing with existing antibiotics. Such strategies could effectively counteract the emergence of resistant bacterial strains, providing a robust defense against bacterial infections.

Keywords: *Pseudomonas aeruginosa, antimicrobial peptide, efflux pumps, proteomic*

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Target Discovery of Natural Myrianthic Acid Through Label-Free Proteomics and Mass Spectrometry Approach

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Due to their diverse structural characteristics, natural products (NPs) serve as a rich source for potential drug candidates, offering significant advantages in terms of application and biocompatibility. Unravelling the protein targets of these compounds is pivotal for understanding their mechanisms of action and facilitating the development of novel drugs. Chemical proteomics has emerged as a critical tool in this domain, offering various strategies for identifying NP protein targets.

This work explores the intricate interplay between myrianthic acid (MA)[1], a natural triterpenoid with ursane skeleton extracted from *Oenothera maritima* Nutt. (Onagraceae), and its possible protein counterparts. It employs cutting-edge MS-based chemical proteomic techniques, such as Drug Affinity Responsive Target Stability (DARTS)[2] and targeted Limited Proteolysis coupled to Mass Spectrometry (t-LiP-MS)[3], to successfully elucidate the complex molecular interactions underlying MA's biological activities.

The discovery of fatty acid synthase (FAS) as a significant MA target marks a significant breakthrough in natural compound-protein interactions. Through meticulous experimentation and validation procedures, the study elucidates the MA/FAS complex. Furthermore, *in vitro* assays showcasing MA's inhibitory effects on FAS enzyme activity underscore its potential therapeutic relevance, particularly in combating tumor proliferation.

Given FAS's pivotal role in various pathological conditions, notably cancer, MA chemical moiety holds promise as a foundational framework for the development of targeted therapeutic strategies. Moreover, the comprehensive understanding of myrianthic acid's mode of action against FAS opens avenues for the design and optimization of novel small-molecule inhibitors with enhanced efficacy and selectivity, heralding a new era in precision medicine approaches for combating debilitating diseases[4].

Keywords: *drug discovery, functional proteomics, fatty acid synthase, t-LiP, DARTS, pre-clinical investigations*.

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Molecular docking and UV-Vis analysis of 3-O-Methylfunicone β-cyclodextrin complex for antiviral drug development mechanism

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The research on new antivirals is aimed to obtain safe and innovative drugs. In animal organisms the agonists of aryl hydrocarbon receptor (AhR) influence the immune response to various viral infections, including Coronavirus diseases. AhR antagonists counteract CoV infection in mammalian cells. Funicones represent a homogeneous group of fungal polyketides which possess important biological properties that promote their consideration as potential antivirals[1]. Our study is focused on a particular funicone called 3-O-methylfunicone (3-OMF). This secondary metabolite, extracted from *Talaromyces pinophilus*, showed antiviral activity against canine coronavirus CCoV and bovine herpesvirus inhibiting AhR[2]. However, 3-OMF shows a low solubility, a characteristic that could negatively impact its use as active antiviral agent.
Cyclodextrins (CDs) can be useful to improve the pharmacokinetic of 3-OMF. In fact, CDs are oligosaccharides known for their complexing capacity, low toxicity and solubility[3].
We report the analysis of the interaction in solution between 3-OMF and β-cyclodextrin (β-CD). We followed the 3-OMF- β-CD host-guest inclusion properties by UV-Vis, evaluating stoichiometry and binding constant of the formed complex, and by molecular docking analysis.

Keywords: *funicone, complex, cyclodextrins, drugs.*
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Innovative Formulations for Agricultural Applications

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Over time the world's population continues to grow [1], resulting in an increased demand for food and an intensification of agricultural practices. The extensive use of pesticides and fertilizers is a largely applied strategy, despite the drawbacks it brings like soil contamination, food pollution, and groundwater pollution [2]. In the agritech field the newest fertilizers and pesticides formulations are based on engineered nanomaterials. This innovative technology allows for the development of smart carriers designed for the delivery of fertilizers and pesticides to plants. By introducing chemical modifications, a smart carrier can be engineered to release the active ingredients (AIs) only upon stimulation from specific sources such as light, pH, temperature, or enzymes [3]. The transportation of AIs exclusively to the plant, triggered by an external stimulus, can lead to a reduction in soil and water pollution by minimizing the leaching.
The employment of lignin as a bio-based surfactant represents a promising strategy in the creation of environmentally friendly smart delivery systems. This method is focused on lignin's amphiphilic characteristics, allowing for molecular self-assembly. In particular, the main lignin building blocks (p-coumaryl alcohol, coniferyl alcohol, sinapyl alcohol) contain an aromatic ring acting as the hydrophobic segment and hydroxyl groups acting as the hydrophilic segment. An eco-friendly strategy may involve the utilization of agricultural food waste to develop novel lignin-based carriers for fertilizers and pesticides. Particularly, lignocellulose, a complex material found in agricultural waste, has emerged as a promising solution to meet the growing demand for eco-friendly compounds in the agricultural sector. The application of lignocellulose extends to the production of smart pesticides [5], which are known for their biodegradability and non-toxic nature. The introduction of lignocellulosic smart carriers in agriculture has the power to transform the industry, facilitating the adoption of a circular economy model. This approach involves recycling agro-wastes to develop pesticides or fertilizers, ultimately boosting agricultural output and consequently increasing waste production. The circular model offers immense potential for utilizing lignocellulose as a sustainable surfactant in agricultural formulations, promoting bio- and eco-friendly practices.

Keywords: *lignocellulose, lignin, responsive carrier, formulation, sustainable agriculture*

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Metal-free synthesis of selenoglycosylated eumelanin monomers

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Eumelanins are a class of dark, insoluble biopigments arising by the oxidative polymerization of 5,6-dihydroxyindole (DHI) and 5,6-diacetoxy-indole-2-carboxylic acid (DHICA) and related derivatives, involved in important biological functions (*e.g.*, homeostasis, photoprotection)[1]. Due to their natural origin and peculiar physical-chemical properties, eumelanin can play a key role in biomedical applications and bioelectronics[2].
In a previous study, the first water-soluble eumelanin monomer 3-thiogalactoside-DHI (S-GAL-DHI) allowed noteworthy insights in structural investigations of DHI melanin[3]. In the view of the emerging interest of soluble glycosylated eumelanin in materials science and in glycobiology, the synthesis of new types of glycosylated melanin involving diverse saccharide precursors represents a field of research of high relevance.
In this work, a series of 3-selenoglycosylated eumelanin derivatives were prepared through a practical and efficient approach exploiting a metal free, simple, and scalable synthetic route. The strategy is feasible for installing both mono- and disaccharide units on on both O-protected eumelanin precursors 5,6-diacetoxyindole (DAI) and 5,6-diacetoxyindole-2-carboxylic-acid (DAICA) and relies on the exploit of glycosyl diselenides in the presence of *N*-halosuccinimide and tetrabutylammonium halides.

Keywords: Glycosyl selenides; Dihydroxyindoles; Eumelanins
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Il Contributo dei Giovani Chimici in Campania
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Campanian particulate matter source apportionment

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In recent decades, great attention has been paid to **Particutate Matter** (**PM**) by the scientific community due to the correlation between fine PM exposure and adverse health effects(1). Among the airborne pollutants, **Lead** (Pb) is one of the most widespread and toxicologically effective(1). Metallic Pb and inorganic Pb compounds are classified as possible carcinogenic to humans; Pb can bioaccumulate in the human body system, causing damage to human nervous system, cardiovascular diseases, reproductive impairments and catalyzing cells oxidative stress (1).

In Italy, Legislative Decree no. 155 of 13/08/2010 (implementation of European Directive 2008/50/EC) defines the atmospheric PM10 and airborne pollutants threshold concentration values; these threshold values are exclusively applied for anthropogenic particulates.

The aim of the research work is to develop an analytical method for determining lead isotopic ratios in PM10 by means of **Double Focusing Multicollector ICP-MS** and apply it to samples from the Campania region.

Pb has four stable isotopes, ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. The measurement of its **isotopic ratios** $\left(\text{i.e. } \frac{204_{Pb}}{206_{Pb}}, \frac{207_{Pb}}{206_{Pb}}, \frac{208_{Pb}}{206_{Pb}}\right)$ can act as particle "fingerprint" giving an insight in their origins (crustal, vehicular traffic, municipal solid waste incinerator, etc …).

Defining Pb origins help the detection of PM10 sources. Therefore, when the atmospheric concentration of these particles exceeds the threshold value (according to the Legislative Decree no. 155 /2010), the isotopic information enables to distinguish natural or anthropogenic causes.

Besides, outlining the Pb isotopic pattern in PM10 allows to define the contribution of different Earth's surface sources to the total amount of Pb detected in this particle fraction. So, recognizing Pb sources is an effective tool for controlling the concentration of this pollutant in the atmosphere.

The PM sampling is carried out by ARPAC monitoring network, using high-volume samplers placed in Campania (Italy) environmental interest points (urban centers, busy roads…). The sampling flow is 2.36 m^3/h, single sampling time is 24 hours and the use of quartz fiber filters Ø = 47mm is provided (according to the technical standard UNI EN 12341:2014).

Particles morphology and chemical composition, characterizing aerosol diffusive properties(2) and health impacts(3), constitute complementary information to isotopic analysis. Therefore, additional morphological and elemental analysis will be performed using Scanning Electron Microscopy Energy Dispersive X-ray Analysis (**SEM-EDX**) and an in-air millibeam Particle-induced X-ray emission (**PIXE**) setup. Both techniques are non-destructive and don't require sample preparation or extraction, thus reducing the contamination from chemical reagents.

Keywords: Source apportioning, particulate matter, Lead, atmospheric pollution.

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Photodegradation kinetics of ibuprofen promoted by Fe-CeO_2 catalysts active under visible light

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Ibuprofen is one of the most widely used pharmaceutical products in the world, first introduced in England in 1967 as an anti-infiammatory, analgesic and anti-pyretic[1]. Due to its use and resistance to biodegradation, ibuprofen is considered an environmental pollutant.[2] In particular, is considered an emerging pollutant, that is a pollutant whose effect on the environment and human health is not yet fully understood, so it is not yet covered by water quality regulations.[3] Existing waste water treatment plants are not currently capable of removing all existing pharmaceutical compounds and therefore their effluents are a major source of input of pharmaceuticals in aquatic ecosystems. An effective method for ibuprofen's removal is the catalytic photodegradation, which is part of advanced oxidation processes (AOPs); their effectiveness depends on the generation of reactive free radicals which are able to oxidize and decompose numerous organic compounds.[4] Cerium oxide and materials containing CeO_2 have been subject of great interest as catalysts promoters of such processes with UV radiation but, its photocatalytic activity can be increased by allowing the light to be absorbed in the visible region. This can be considered a novelty of great interest since more than 40% of the incident sunlight consists of visible light, while only 4-5% is of ultraviolet radiation. Therefore, extending the absorption range for photo-catalysts to this region allows the use of solar radiation as a natural source of energy for photocatalysis.[5] Using iron as a doping metal, catalysts with superior catalytic performance are obtained, due to the consequent decrease of the band gap and absorption in the visible region.
The objective of the master thesis work was the study, under laboratory conditions, of the catalytic photodegradation of ibuprofen in aqueous solution, using as catalysts Fe-CeO_2 with different percentage of iron, to choose the optimal catalyst for the reaction. Further objectives of the thesis work were the kinetic study with the chosen catalyst, aimed to calculating the kinetic parameters, and the identification of the degradation products resulting from the reaction. The catalyst found to be the most active in the process of photodegradation of ibuprofen, with radiation in visible range, was the one with iron percentage 2.5, which produces greater removal of ibuprofen than adsorption, another phenomenon verified in the use of such catalysts during experimental tests. As a degradation byproduct, p-isobutylstyrene was detected, in concentrations initially increasing, and then decreasing, suggesting further degradation of the formed byproduct, a positive aspect noted during the test performed.

Keywords: *Ibuprofen, Photodegradation, Fe doped CeO_2, Visible light*

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Invasive seaweeds and their hybrids as natural sorbent for wastewater remediation

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The presence of emerging pollutants and their negative impact on the environment has aroused interest, so many wastewater treatment approaches have been developed to remove pollutants but not all of them are effective. Among the many options, adsorption still represents a promising technology possibly scalable on an industrial scale level. The process based on the use of solid sorbents is considered a convenient choice because of its low execution costs, ease of operation, simplicity of design and fast adaptation to variable pollutant concentration. Given these potentialities, several commercial materials have been extensively investigated as adsorbents in water pollution control, but the interest in the use of biomass-based adsorbents is constantly increasing. Natural materials are generally preferred as adsorbents due to their low cost and wide availability. The production of functionalized natural materials which have a better capture capacity and speed is also arousing great interest [1]. The use of macroalgae as there are has been considered as a potential alternative for pollutant removal [2,3] opening the way to the development of new biomass-derived adsorbents.
In this contribute we report the results of methylene blue (MB) adsorption tests performed by using as adsorbents two types of invasive seaweed: *Caulerpa cylindracea* (an invasive green alga in the Mediterranean Sea where it was unintentionally introduced in the early 1990s) and *Asparagopsis taxiformis* (a species of red algae, distributed in both tropical and in warm temperate waters). The two seaweeds were tested toward MB adsorption as this dye is used extensively in cotton, textile and wood industries and it can lead to the development of diseases. In order to expand the field of application of these materials (e.g. easy recover after use and possibility to undergoes to multiple adsorption cycles) we produce hybrids with magnetic particles directly grown on the macroalgae surface. The two seaweeds and their hybrids were characterized by proximate analysis, ultimate analysis, ICP-MS analysis and thermal analysis. The effects of MB concentration, contact time, and adsorbent mass of the materials were investigated to obtain kinetics parameters. Furthermore, the cyclability of the materials was also evaluated after the first uptake evidencing perspectives and shortcomings.

Keywords: *Seaweed, waste valorization, adsorption kinetic, Methylene blue uptake.*

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A printed electrochemical strip to evaluate nanovectors encapsulation

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Lipid nanocarriers (LNCs) are powerful tools for drug delivery, used in various medical applications from cancer treatment to vaccines [1]. However, challenges remain in optimizing drug encapsulation efficiency and minimizing loss during the process. Techniques like chromatography, mass spectrometry, and UV-Vis absorption are commonly used to evaluate encapsulation effectiveness during formulation development [2]. In this work, we present an innovative, portable, easy-to-use, and inexpensive strategy to rapidly assess LNC quality. The approach involves using an electrochemical strip with a screen-printed electrode to detect methylene blue (MB) as a representative cargo encapsulated in various liposome models (distearoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and hydrogenated soybean phosphatidylcholine). The experimental setup, including MB release and electrochemical detection, was optimized using a multivariate design of experiments (DoE) with a d-optimal design. This allowed optimizing 4 variables with only 19 experiments. Additionally, principal component analysis and linear discriminant analysis (PCA-LDA) were used to distinguish the lipid bilayer compositions of the liposomes by analyzing their voltammetric profiles.
We highlight the synergy between portable electroanalysis and multivariate analysis as a powerful tool for enhancing quality control in pharmaceutical technologies. This approach shows promise not only for LNCs, but also for analyzing naturally occurring lipid nanoparticles like exosomes, which could be valuable in diagnostics.

Keywords: *Lipid Nanocarriers, Screen-printed, Chemometrics, Quality control*

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Binder-pigment interaction by proteomic approaches

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The appearance of a painting is the result of the materials used in terms of the pigments present (which determine the color) and the organic binder (which determines the painting technique).[1] However, paintings are not stable or immovable and reactions take place over time, which strongly influence the final look of a painting. Once applied, the paint undergoes chemical reactions resulting in the formation of a dry film. Further chemical changes occur during ageing, such as oxidation and cross-linking of the organic media, reactions between the paint binder and the pigments as well as reactions of the pigments with the atmosphere.[1] Although modifications arising from binders-pigments interactions may change drastically the appearance and physico-chemical stability of a painting, they have been relatively underexplored, with most research focusing on oil-based media.[1] Notably, very little is known on the nature of the interactions between the inorganic pigments and the proteinaceous binders, and their impact on paint aging under variable environmental conditions.[3]
As an initial approach to this problem, we studied the interaction of casein (proteinaceous binder used in the tempera technique) with four inorganic pigments that are commonly employed in paintings: azurite ($Cu_3(CO_3)_2(OH)_2$), Saint John's white ($CaCO_3$), cinnabar (HgS) and red lead (Pb_3O_4). Paint reconstructions had been prepared by applying pigments mixed with water solutions/dispersions of casein onto glass slides. The resulting paint films had been artificially aged to simulate the condition of historical paintings and then scratched to obtain fine powders. We used proteomics approaches based on Nano LC-MS/MS to analyze the chemical modifications undergone by proteins as an effect of ageing, and depending on the pigment.[4] Additionally, we employed a Lip-MS-based approach, novel to the cultural heritage field, to examine how pigments can influence the 3D structures of proteinaceous binders. This technique enabled the identification of specific protein regions possibly affected by protein binder-pigment interactions.[5]
By shedding light on the chemical interactions between pigments and proteinaceous binders, our research provides valuable insights into the aging processes of historical paintings. This knowledge can inform conservation strategies, helping to preserve the original appearance and structural integrity of these works of art for future generations.

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Keywords: *Binder-pigment interaction, Ageing, Nano LC-MS/MS, Lip-MS*

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Metal–Organic Framework-derived ZnO with Enhanced Ethanol Sensing Properties

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The impact of volatile organic compounds (VOCs) on human health is being taken more and more seriously as concerns about public health continue to grow. Among VOCs, ethanol is a colorless, volatile, odorous, and flammable liquid that can cause a variety of health problems after repeated exposure, including headaches, fatigue, and brain damage [1]. Ethanol gas sensors are required in several industries, including environmental protection, industrial production, and vehicle safety. Chemiresistors have emerged as potential candidates for VOC sensing due to their simplicity in construction, wide range of acceptable sensitive materials, and straightforward sensing data [2]. The fundamental component of chemiresistive sensors is an interdigitated electrode (IDE) with a sensing layer on top. Metal oxide gas sensors have gained importance in recent years as sensing layer because they can detect the presence and concentration of gases via electrochemical processes. As an n-type semiconductor, ZnO plays a significant role in gas sensing due to its great chemical stability, rapid recovery, high sensitivity to various gas molecules, simplicity of manufacture, and easy doping cost [3]. However, there are some aspects that need to be improved such as selectivity, operating temperature, and limit of detection. The gas sensing properties can be improved by altering the structure and one approach is to design special structures with various morphologies. Metal-organic frameworks (MOFs) have garnered significant interest in the sensing field, because of their intriguing properties, which include large surface area, high porosity, tunable morphologies, and structural diversities [4]. However, most MOFs have a low electrical conductivity. To enhance this characteristic, MOFs can be hybridized and mixed with both organic and inorganic components to produce composite materials or they can be transformed into more stable structures. After calcination, MOFs are converted into metal oxides with well-designed structures. It is also possible to calcinate noble metal functionalized MOF to produce the corresponding doped metallic oxide enhancing the sensing performance. In this work, zinc-based MOF structures belonging to the ZIF family were synthesized, structurally characterized, and calcinated. The resulting ZnO was then assembled in a chemoresistive architecture. The sensors were tested in a humidity-controlled chamber with the possibility to heat the sensor and expose it to ethanol concentrations ranging from 0 to 100 ppm.

Keywords: *sensing*, *ZnO, Metal–organic frameworks, Gas sensors, Volatile organic compounds.*

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Identification of 1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-based compounds as new BRD9 binders

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Bromodomains are druggable protein modules that recognize post-translational modifications (PTMs) on histones and play roles in various diseases, including cancer and inflammation.[1] Among them, Bromodomain-containing protein 9 (BRD9), a key component of the mammalian SWI/SNF chromatin remodelling complex, has emerged as a crucial target, especially in leukaemia.[1] Supported by the Italian Association for Cancer Research (AIRC),[2] we have been recently involved in the discovery of novel modulators of this protein. In light of this, we here present the discovery of novel BRD9 binders featuring the 1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine chemical motif. Through a virtual screening approach and utilizing a 3D structure-based pharmacophore model previously developed by us,[3] our *in silico* investigation led to the identification of a promising initial hit (compound **1**, Figure 1). The encouraging biophysical results for compound **1** inspired further structural modifications at the C-4 and C-6 positions of the central core. Consequently, we designed and synthesized a series of 19 derivatives (compounds **2**–**20**) to thoroughly investigate the chemical space at BRD9 binding site. Among these, four compounds (**5**, **11**, **12**, and **19**, Figure 1) emerged as potent BRD9 ligands, exhibiting IC_{50} values in the low-micromolar range. Moreover, compound **5** demonstrated promising antiproliferative activity *in vitro* against HeLa and A375 cancer cell lines and a safety profile on healthy cells compared to the known inhibitor (I-BRD9).

Keywords: Drug discovery, Bromodomains, Computational techniques, Synthesis, Biophysical assay.

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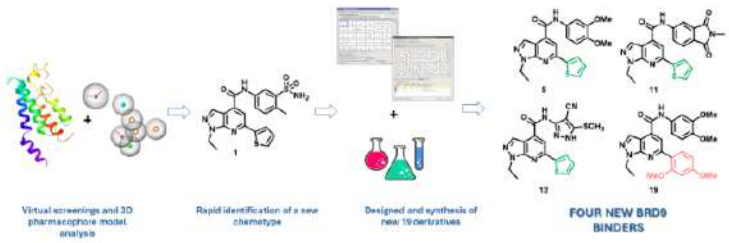

Figure 1. Scheme of the multidisciplinary approach used for the identification of novel BRD9 binders.

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Validation of analytical methods for trace elements determination in biological matrices

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Fifty years ago, the World Health Organisation defined *health* as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". Environmental pollution is now a ubiquitous and widespread phenomenon, and it is part of the environment in which humans live. The European Environment Agency (EEA) defines as pollutants all those substances that, when introduced directly or indirectly into the environment, produce harmful effects on it and on the health of living beings. Contaminants of chemical origin can be divided into two main classes: organic and inorganic pollutants. Among inorganic pollutants, the main ones to mention are arsenic, cadmium, chromium, mercury, and lead, as well as some compounds of nitrogen, phosphorus, oxygen, sulphur and silicon, and some minerals such as asbestos.

In 2005, the concept of the "exposome" was outlined as "the totality of environmental (*i.e.*, non-genetic) exposures to which an individual is exposed from conception onwards". Its role is to understand how the totality of an individual's exposures over a lifetime can affect human health because the etiology of a health condition can rarely be explained by a single exposure. [1] These substances, with which human beings coexist daily, can develop their toxic action when taken by the body in doses above tolerable levels, giving rise to bioaccumulation processes in the body, causing more or less extensive damage, and in the most serious cases even leading to the death of the individual.[2]

Human beings and their state of health are therefore considered important indicators of the state of the environment, and the determination of contaminants within biological matrices makes it possible to indirectly assess the quality of the environment.

Biological matrices of analytical interest are blood, blood serum, urine, hair, seminal fluid, etc. Each of them stores different traces of pollution that are capable of being retained for different periods of time, and therefore the determination of heavy metals and other trace elements in these matrices is an important *tool* of characterizing environmental pollution and consequently for assessing possible negative effects on the health of the population.

The blood matrix, along with plasma and blood serum, are examples of "stable" matrices as they exhibit less variability than other matrices, such as nails and hair, which retain traces of pollution for shorter periods of time.

As part of the FASt and ExpoMap projects, using as a starting point and reference the method described by the Istituto Superiore di Sanità in the ISTISAN 15/30 Report, an analytical method was validated for the determination of trace elements (Hg, Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Sn, Sb, Te, Ba, Pb, U) in blood serum, seminal fluid and urine.[3]

The validation of the method involved treating the samples by means of microwave-assisted oxidative acid digestion, while for the quantitative determination of the elements under analysis, the technique adopted was Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), as the elements considered occur in very low concentrations within the biological matrices under investigation.

The performance obtained by the validated methods met the requirements necessary for the validation of a method, as defined by the Eurachem Guideline "Fitness Purpose of Analytical Methods", proving to be fast and efficient when applied to different case studies, within research projects aimed to assess the impact that the environment has on human organism.[4]

Keywords: *biological matrices, trace elements, method validation, ICP-MS*

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Gold nanoparticles supported on Poly(2,6-dimethyl-1,4-phenylene oxide) as catalyst: from oxidation processes to hydrogen production

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Catalysis using metal nanoparticles is a current research topic. Gold nanoparticles (AuNPs) promote several organic transformations under mild and green conditions, with high selectivity and activity.
A large number of inorganic and organic supports have been used to prevent the coalescence of the nanoparticles and preserve their catalytic activity.
Recently, porous organic polymers (POPs) have attracted significant interest as metal nanoparticle support because of their ease of synthesis and functionalization, low cost, stability, and extensive potential for reuse.
In this contribution, we report a catalytic system based on gold nanoparticles incarcerated in a poly(2,6-dimethyl-1,4-phenylene oxide) (PPO) matrix. PPO is cheap and commercially available and has been recently recognized as a polymorphic nanoporous polymer that efficiently absorbs small organic molecules from air or water, even at trace levels. [1] The AuNPs-PPO catalyst (2%w of Au) was readily prepared by co-precipitation in methanol of the *in situ* prepared AuNPs and PPO.[2] Spherical nanoparticles of 7 nm were characterized by WAXD, SEM, and TEM analysis. We explored the potential of the catalytic system in two different and interesting catalytic processes: the aerobic oxidation of alcohols into carbonyl compounds and the decomposition of formic acid to obtain clean dihydrogen. For both reaction processes, the role of the polymer matrix, the reaction conditions, and the recyclability of the catalytic system were investigated.

Keywords: gold, nanoparticles, oxidation, hydrogen

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Multi-level proteomics defines multiple unbalances in methylmalonic acidemia

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Methylmalonic acidemia (MMA) is a rare inborn error of metabolism due to mutations in the methylmalonyl-CoA mutase (*MUT*) gene, whose product catalyzes the mitochondrial conversion of the methylmalonyl-CoA into succinyl-CoA for Krebs cycle functioning. MMA patients' body fluids show toxic metabolites accumulation, such as methylmalonic acid and methylcitric acid, due to aberrant degradation of odd-chain fatty acids, cholesterol, and branched-chain amino acids. Despite the prompt diagnosis of MMA is feasible owing to mass spectrometry-based newborn screening, actually the cell damage and stress mechanisms in MMA are not fully clarified and the current therapies are ineffective [1-2]. To shed light on MMA pathogenesis and disease mechanisms, the proteomic landscape of diverse MMA cell models was investigated. First, a MUT-knockout (MUT-KO) cell line was generated via CRISPR/Cas9 in HEK-293 cells; then, dermal fibroblasts derived from MMA patients were collected and cultured.
Multi-proteomic analyses were performed on the MMA models to characterize the whole cell proteomes and their sub-proteomes [3-6]. Global proteome analysis showed alterations in autophagy- and lysosomal-related pathways, demonstrating a strong compromission of the lysosomal/autophagic machinery in MUT-deficient cells that are not able to fully degrade accumulated matter. Both the cell models showed profound dysregulations in the mitochondrial routes. In fact, the analysis of the mitochondrial proteome revealed enrichment of specific pathways related to fatty acid and carbohydrate metabolism, suggesting unbalances in energetic metabolism. Finally, the profiling of the nuclear proteome revealed novel terms related to epigenetic and structural modifications, never previously connected with MMA. These data suggest that multi-level dysregulations occurring in MMA patients trigger the cellular and organ damage, providing hints towards novel potential pathways to be targeted to develop efficient therapeutic intervention in MMA.

Keywords: *proteome analysis, multi-proteomics, metabolic disease, methylmalonic acidemia*

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Probing FPPS enzyme inhibition: a multidisciplinary approach

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Farnesyl pyrophosphate synthase (FPPS), a key enzyme in the mevalonate (MVA) pathway, catalyzes two sequential condensation steps to produce farnesyl pyrophosphate (FPP), required for the post-translational prenylation of small GTPase proteins. Protein prenylation is crucial for the localization of these proteins to cell membranes and, therefore, for their biological function. Thus, inhibition of FPPS represents a novel potential strategy to downregulate the activity of mutated RAS superfamily GTPase proteins, which are known drivers of oncogenesis.[1-3] Here, we present a methodological approach for the design of peptides probing FPPS, combining computational strategies and experimental procedures. Using molecular docking and ^{1}H nuclear magnetic resonance (NMR) experiments based on Saturation Transfer Difference (STD) and enzymatic NMR assay and Surface Plasmon Resonance (SPR), we found three peptides with the ability to bind FPPS and modulate its catalytic activity. Additionally, results obtained on MC38 (murine colon adenocarcinoma) cells using live imaging technologies, such as the IncuCyte Live-Cell Analysis System, confirmed the significant ability of the designed peptides to attenuate cell proliferation, thereby anticancer activity. Finally, genomics and metabolomics profiling allowed to unravel the possible mechanisms underlying the activity of the peptides, confirming their involvement in the modulation of the MVA pathway.

Keywords: *FPPS, isoprenoids, peptide ligands.*

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Dimer optimization of G-quadruplex-forming aptamers

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High-Mobility Group Box 1 (HMGB1) is an abundant, highly conserved, non-histonic nuclear protein present in almost all eukaryotic cells[1,2]. In an inflammatory state, HMGB1 is actively secreted from immune cells in the extracellular matrix, where it behaves as a proinflammatory cytokine[3], contributing to the pathogenesis of various chronic inflammatory and autoimmune diseases as well as cancer[4]. Considering the ability of this protein to induce bending in double-stranded DNA[5,6], as well as the identification of HMGB1 as a telomeric and non-telomeric G-quadruplex (G4)-interacting protein[7,8], in a recent work we identified a set of G4-forming aptamers from a focused library of G-rich oligonucleotides able to interact with high affinity with the protein and also inhibit the HMGB1-induced cell migration[9]. A more in-depth biophysical and biological characterization of one of the best anti-HMGB1 aptamers - that we named L12 - revealed that its efficacy was mostly due to its ability to spontaneously form dimeric species. Thus, the aim of this work is the design, synthesis and evaluation of the biophysical properties of covalent dimers of the anti-HMGB1 G4-forming aptamer L12, chosen as a model compound of the series. These novel dimers are obtained by connecting two L12 sequences through different linkers with variable length and flexibility in 5'-3', 3'-3' and 5'-5' orientation, so to develop optimized constructs that can bind HMGB1 with higher affinity and better inhibit the protein pathological activities.

Keywords: *HMGB1, aptamer, G-quadruplex, cancer*

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CYCLIC PEPTIDOMIMETICS AS SUPPRESSOR OF CYTOKINE SIGNALLING 1 (SOCS1)

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In response to cytokine stimulation in immune, inflammatory and carcinogenic processes, the Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway is upregulated [1]. This pathway is endogenously modulated by the Suppressor Of Cytokine Signaling (SOCS) family of proteins through a negative feedback mechanism [1]. The SOCS family comprises eight members, but only SOCS1 and SOCS3 possessing a small protein region known as KIR (Kinase Inhibitor Region) in their N-terminal region and that is crucial for inhibition of JAKs' kinase activity [2]. In particular, these proteins exert a dual inhibition mechanism: the first, common to the other SOCS members, uses the SH2 domain to compete with the SH2 of STATs; the second is thanks to KIR, which is able to interact directly with JAK2 by acting as a pseudosubstrate [2]. The development of SOCS1 mimetics employing KIR linear peptide outlined its anti-inflammatory properties [3]. More recently, cyclic peptidomimetics of KIR peptide containing a lactam bridge demonstrated to efficiently inhibit JAK-STAT closely mimicking the biological activity of the entire SOCS1 both *in vitro* and *in vivo* [4]. Here, we present novel cyclic analogues obtained using the Chemical Linkage of Peptide onto Scaffold (CLIPS) strategy to create non-native linkages between thiol side chains of cysteines and xylene scaffolds, named thio-monocycle PS5(Nal1) and thio-bicycle PS5(Nal1) [5]. The affinity of the thio-analogues for JAK2 was assessed by MicroScale Thermophoresis (MST) and phosphorylation inhibition assays, their serum stability by liquid chromatography-mass spectrometry (LC-MS) analysis, and their structural properties by Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) [5]. In conclusion, presented results demonstrated that these bioactive sequences are particularly suitable and versatile for *ad hoc* chemical modifications required for the translation of the KIR-SOCS1 peptidomimetics, into specific inhibitors of JAK2.

Keywords: *cyclic peptidomimetics, JAK/STAT, SOCS1, CLIPS*
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Circular oligomers formed by Ros/MucR family members act as mediators of DNA condensation in α-proteobacteria.

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The transcriptional regulator MucR from *Brucella* species controls the expression of virulence and many others genes by binding to AT-rich DNA. MucR and its homologs constitute the Ros/MucR family, whose members occur in α-proteobacteria [1]. Recently, MucR has been classified as a new histone-like nucleoid structuring protein (H-NS) [2]. Despite the lack of sequence homology, MucR shares many functional similarities with H-NS and H-NS-like proteins, which play a role in structuring the bacterial genome and act as global transcriptional regulators. In this study, we combined cryo-electron microscopy (cryo-EM), nuclear magnetic resonance (NMR), and structural modeling approaches to define a structural model of the oligomers formed by MucR and its homolog Ml5 from *Mesorhizobium loti*. Our data reveal that MucR and Ml5 constitute a distinct type of H-NS-like proteins, as their circular quaternary structure differs from that found in other DNA structuring proteins. The ability of Ros/MucR family members to oligomerize and bind AT-rich DNA targets was also explored by molecular docking simulations. Our study sheds light on the mechanism by which Ros/MucR family members bridge and condense DNA, providing insights into the interplay between nucleoid structure and transcriptional regulation in α-proteobacteria.

Keywords: *Ros/MucR family, H-NS, DNA condensation*

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Metabolomic Profiling of Fecal Samples from Breastfed Late Preterm Infants Compared to Those Fed Postbiotic-Supplemented Formula Milk

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In contrast to conventional foods, functional foods provide physiological benefits and can reduce the risk of chronic diseases beyond basic nutritional functions, such as maintaining gut health [1]. Exclusive breastfeeding is recommended from birth to 6 months as the standard for infant nutrition. Human milk contains various nutritional and bioactive components. However, when breast milk is unavailable or insufficient, formula milk is used as a substitute. Infant formula can be enhanced with postbiotics to support the development of immune, metabolic, and microbial systems, like breast milk. Postbiotics are preparations that include microbial constituents and their metabolites produced during fermentation [2]. This study aimed to identify differences in the faecal metabolome of newborns fed with breast milk, standard formula milk, or formula milk supplemented with 0.5 mL of SMART D3 MATRIX (Vitamin D3 10 mcg 400 I.U., Immunofos (fermented FOS from *Lactobacillus paracasei* strain CNCM I-5220) 20 mg), using untargeted and targeted MS-based metabolomics followed by multivariate data analysis. A prospective single-centre observational cohort study was conducted from March to December 2022 at Buon Consiglio Fatebenefratelli Hospital (Naples, Italy). The study enrolled 27 late preterm newborns with birth weights appropriate for gestational age. The participants were divided into three groups: group A (formula + SMART D3 MATRIX, 7 infants), group B (standard formula, 9 infants), and group C (breastfeeding, 11 infants). Stool samples for metabolome analysis were collected at T0 (5-7 days after birth), T1 (1 month old), and T2 (3 months old), resulting in 81 samples, which were extracted and analyzed using liquid chromatography coupled with high-resolution mass spectrometry in duplicates. The raw LC-MS data from T0, T1, and T2 were processed using MZmine 2.53 and SIMCA P 17.0 (Umetrics AB, Umea, Sweden) for Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA).
The analysis revealed good separation between stool samples at different time points (T0, T1, and T2), showing similarities between the faecal metabolomes of breastfed infants and those fed formula plus postbiotics. Based on these findings, this new formula milk could be considered a viable alternative to breast milk [3].

Keywords: *Mass spectrometry-based metabolomics, breast milk, formula milk plus post biotic*
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Compatibilization of isotactic polypropylene (iPP)/polyeathylene (PE) blends with iPP-*graft*-PE copolymers

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Polyethylene (PE) and polypropylene (PP) are the two most common plastics in the world; the main issue associated with them is the impossibility of recycling mixed wastes of them due to their immiscibility. In recent years, research attention has focused on the use of compatibilizers to improve the miscibility of iPP and PE.[1,2] This work reports the synthesis, characterization and analysis of the compatibilizing capability of iPP-*g*-HDPE graft copolymers for PE/iPP blends.
The synthesis was carried out in two steps: production of PE macromonomers with allylic termination and subsequent synthesis of iPP-*g*-PE graft copolymers. In order to ensure an effective compatibilizing action, the graft copolymers were specifically designed to have PE graft and iPP spacer lengths greater than the critical entanglement lengths M_e of the neat PE and iPP species ($M_e \approx$ 1-2 kDa for PE and $M_e \approx$ 6 kDa for iPP).[3] Through analysis of GPC traces and NMR spectra, high reproducibility of the synthetic methodology results was found.[4]
The nomenclature adopted to identify the samples is ${}^{w}iPP_x$-g_n-PE_y where: w is the number average molecular weight (M_n) of the entire graft copolymer, x is the spacer length, n is the number of grafts per chain, and y is the molecular weight (M_n) of the macromonomer. Four samples were analyzed: two iPP-*g*-HDPE with short branches, i.e. y = 3.6 kDa and n = 10 and n = 14, and two samples with long branches i.e. an iPP-*g*-HDPE with y = 13 kDa and $n \approx 9$ and an iPP-*g*-LLDPE with y = 11 kDa and n = 25.
Small amounts (1-3 wt%) of the graft copolymers were added to HDPE/iPP mixtures having a composition similar to that found in landfills (70/30 wt/wt). It is shown that the resultant blends show outstanding mechanical properties compared with those of the neat to HDPE/iPP mixtures at the same (70/30 wt/wt) composition. The best compatibilization effect occurs for the iPP-*g*-HDPE graft copolymer with long HDPE branches. A possible mechanism of compatibilization is proposed.

Keywords: *isotactic polypropylene, polyethylene, compatibilization, olefin graft copolymers*

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Synthesis of new organocatalysts based on cyclic peptoids

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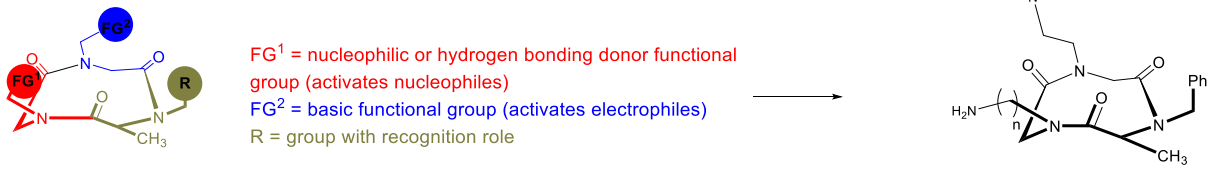
Organocatalysis has become increasingly central to organic synthesis over the past 20 years. It exploits small organic molecules also derived from natural sources as catalysts, thus lowering costs and increasing the green aspect of the reaction as it avoids use of metals. In addition, asymmetric organocatalysts give the possibility of obtaining molecules in an enantioenriched form, which is also a crucial aspect for the synthesis of a variety of drugs that exhibit only one active enantiomer. However, still there are limitations, mainly given by the loading of catalyst needed to perform the reaction and the reusability of the catalyst. Therefore, to date, research toward the synthesis of new catalysts is still open. Valid candidates as potential new organocatalysts could be cyclopeptoids. Cyclopeptoids are the cyclic form of peptoids, which are N-substituted glycine oligomers. Cyclization is known to make structures conformationally more rigid and it was observed that the intrinsic rigidity of their framework can evoke conformational isomerism and conformational chirality, organizing the N-alkyl side chains in stereodefined spatial arrangements[1,2]. In 2018 it was also shown that the presence of stereogenic centers on the backbone of macrocycles of peptoid nature blocks the conformation to a single diastereomeric form[2]. In the contest of organocatalysis, the spatial arrangement of catalytically involved group is crucial, because they must have a defined spatial orientation, so, this characteristic of cyclopeptoids makes them interesting as potential asymmetric organocatalysts. With this in mind, the synthesis of new macrocycles of a cyclopeptoid nature functionalized appropriately with a free amine and a tertiary amine as potential bifunctional catalysts is proposed.



Keywords: *organocatalysis, organic synthesis, peptoid*

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Polybenzimidazole-based electrospun membranes for fuel cell application

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Polybenzimidazoles (PBI) are a basic polymer family characterised by an aromatic structure and the presence of an aminated group, which can interact with bifunctional or multifunctional electrophiles. The synthesis reaction for this material was developed in the 1960s and consists of a condensation polymerization process between 3,3'-diaminobenzidine and terephthalic acid [1]. Over the past 20 years, PBI has been widely studied and used due to its ability to interact with strong acids. The impregnation of PBI in phosphoric acid (PA) is the technique mainly used for high-temperature fuel cells (HTFCs) as it makes the material ductile and highly conductive. The acid serves as a proton carrier, facilitating the proton hopping within the membrane, which is crucial for the functioning of the cell [2]. PBI is commercially available as a membrane obtained by a cast solvent method. However, solution-cast phosphoric acid-doped PBI proton exchange membranes have some disadvantages that can negatively affect their long-term durability and performance, including (a) high acid adsorption, which can lead to swelling reducing mechanical stability [1]; (b) a difficult production process, as solution casting requires hazardous solvents and careful control of the production and drying processes to achieve a uniform thickness; (c) mechanical fragility [3]. In this contribution, the development of PBI membranes obtained through the electrospinning technique is proposed. These new electrospun membranes could provide several advantages compared to those obtained by solvent casting. In particular, electrospinning could allow precise control of membrane morphology and thickness: by adjusting parameters such as polymer concentration, solvent composition, and electrospinning conditions, the membrane's fiber diameter, pore size, and overall structure can be tailored to specific requirements.

Keywords: *fuel cells, polybenzimidazole, electrospinning*

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Drug repositioning, a valid strategy for the identification of a new LIFR antagonist

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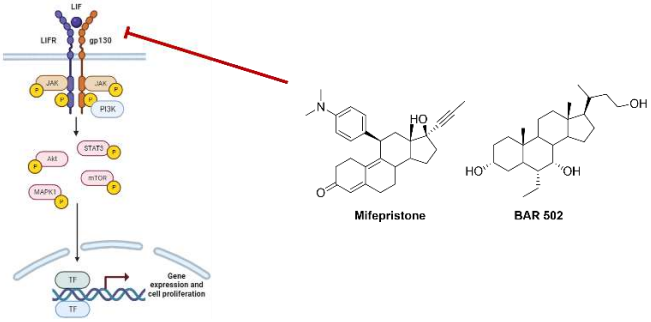
Leukemia inhibitory factor (LIF) is the most pleiotropic member of the IL6 family which plays a prominent role in inflammation, autoimmunity, and cancer. Considering the wide range of activities of LIF, its receptor (Leukemia Inhibitory Factor Receptor, also known as CD118) is currently much studied and it's found in different organs, such as liver, bone, uterus, and the central nervous system. Furthermore, different studies show that LIF promotes the progression and metastasis of tumors, but the mechanism is poorly defined. Its overexpression is correlated with poor prognosis in cancer patients, including in pancreatic ductal adenocarcinoma (PDAC).
PDAC represents the 85% of pancreatic cancer (PC), but 90% of PDAC are detected at an advanced stage beyond the criteria for curative surgery. Most commonly, PDAC patients develop resistance to chemotherapy, so the identification of a new mechanistic molecular pathways represents an urgent need. Since there are no LIFR antagonists available for clinical use, we developed an *in silico* strategy to identify new potential molecules as LIFR antagonists[1]. The results allowed to the identification of mifepristone, a progesterone/glucocorticoid antagonist, as possible LIFR antagonist. Moreover, considering its steroidal scaffold, we also evaluate several bile acids' derivatives in order to identify a new scaffold. These studies have identified BAR 502, a non-bile acid steroidal dual FXR and GPBAR1 ligand, as best candidate[2]. In fact, BAR502 reverses the pattern LIF-induced in a FXR and GPBAR1 independent manner, suggesting a potential role for BAR502 in the treatment of LIFR overexpressing-PDAC. These preliminary studies represent a promising starting point for the identification of a new LIF/LIFR antagonist.



Keywords: *cancer, drug repositioning, steroidal scaffold*

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Methylene blue adsorption from aqueous matrix on geopolymer-based substrates

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In recent years, one of the environmental problems scientists are very interested in, is the contamination of water by "emerging contaminants", due to its seriousness. Among these contaminants, a particular concern compound is methylene blue (3,7-bis(dimethylamino) phenazationium chloride), a common waste from the textile industries that is extremely toxic to human health[(1)]. Geopolymers[(2,3)], are a class of synthetic materials, typically amorphous, aluminium-silicate based, having a structure similar to tectosilicates one, characterized by a three-dimensional network of covalently linked tetrahedral units (AlO_4^{5-} e SiO_4^{4-}). The geopolymers synthesis involves a polycondensation reaction that uses as precursors secondary raw materials such as kaolinite or industrial waste, such as fly ash and blast furnace slag. Geopolymers are obtained by mixing these alumino-silicate powders with a strongly alkaline activating solution based on silicates and/or alkali metal hydroxides. In this context, the following work was developed. Adsorption tests, under thermodynamic conditions, and kinetic tests, in batch and in continuous, were conducted at various temperature and pH conditions in order to understand how these parameters influence the adsorption process. Four different geopolymer samples were used, differing in composition. Samples were washed dried and sieved in order to obtain a specific particle size range. Then samples were conditioned in order to achieve target pH values. A 24h long screening test was run and pH 7 value was chosen. For batch adsorption tests 0.005 g of geopolymer powder, was placed in vials. Here 10 mL variable concentrations of methylene blue solution were added. They were stirred in a thermostatic bath at room temperature, protected from light, for three hours to ensure that the equilibrium conditions were reached. The adsorption isotherms, thus obtained, showed a good affinity between adsorbate and adsorbent and a good uptake capacity of the latter. Kinetic tests were performed in a batch reactor, with the same solution/solid ratio (0.125g of sorbent/250mL of solution) used for the adsorption tests, with fixed methylene blue concentration (30mg/L). From these tests, 3h long, performed for the four samples, it emerged that the adsorption process speed is high and that the sample with best performances is sample A. Tests, with this specific sample, were also performed by varying the following parameters: initial methylene blue concentration, temperature, stirring speed, amount of sorbent. It was seen that no effect of the temperature and stirring speed is recorded, while the adsorption capacity decreases with increasing initial concentration of methylene blue and with decreasing amounts of sorbent. Continuous tests were conducted with steel columns packed with geopolymer (solid geopolymer foam obtained adding Si, in situ, a foaming agent in a mass/mass ratio of 1%) with different flow rate (column used was subjected to numerous washing cycles with water). Methylene blue aqueous solution (4 mg/L) flow rate was adjusted by a peristaltic pump. From adsorption kinetic tests in column, breakthrough curves were obtained it can be seen from that as the flow rate increases, the saturation level of the material packed in it also increases. These encouraging results push us to continue the studies of geopolymer-based materials as substrates with low environmental impact in the adsorption of pollutants from industrial wastewater and in general in the environmental remediation sector.

Keywords: *Geopolymers; adsorption; cationic pollutants; methylene blue; wastewater treatment.*

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Ammonia Decomposition Using a Red Mud-Based Geopolymeric Catalyst for COx-Free Hydrogen Production

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The Bayer process, which involves the caustic digestion of bauxite, generates a waste product known as Red Mud (RM). RM is a mixture of various oxides and carbonates, with its composition largely dependent on the origin of the bauxite. Typically, RM consists mainly of Fe_2O_3, TiO_2, SiO_2, and Al_2O_3. Annually, over 100 million tons of RM are produced worldwide [1], making it a significant challenge to manage due to its high alkalinity (pH 10-13) and the severe issues associated with its storage.
Nevertheless, the high Fe_2O_3 content in RM makes it a promising precursor for developing industrial catalysts, such as those used in ammonia decomposition reactions. Initial studies have shown that, after acid treatment and reduction under a hydrogen flow, RM exhibits good catalytic activity, achieving ammonia conversion rates of 30% [2].
In this project, we synthesized several RM-based geopolymeric substrates under acidic conditions [3] for use as catalysts in ammonia decomposition. These samples vary in chemical composition and morphology, enabling precise control over the content and distribution of the active phase. The catalysts were tested in a flow-through system with a fixed-bed reactor. A mixture of NH_3 and Ar (as an inert gas) was passed through the reactor, and the NH_3 conversion was continuously monitored using a gas chromatograph (GC) with a thermal conductivity detector (TCD).
The focus of this work is to determine the optimal reaction conditions for maximizing ammonia conversion, study the reaction kinetics, and define the kinetic and thermodynamic parameters of the process.

Keywords: *hydrogen production, ammonia, catalyst, fuel cell, red mud, bauxite residual, geopolymer*

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Adsorption properties of a hybrid geopolymer toward Y^{3+} ions

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Yttrium, a rare earth element (REE), is employed in a variety of medical applications and in a range of industrial and technological processes. The dispersion of yttrium(III) ions into the environment results in the exposure of humans and fauna to toxic health effects. The present study examined the removal of yttrium(III) ions from aqueous solutions by adsorption on organic hybrid geopolymer, where the inorganic component is represented by an aluminosilicate and the organic component consists of polydimethylsiloxane[1].The removal efficiency of the target pollutant was evaluated by means of kinetic models and adsorption isotherms. Measurements were conducted at two ionic strengths, 0.1 and 0.01 M $NaClO_4$, at different temperature (25 and 50 °C) and at varying pH (4.0 and 7.0). Potentiometric measurements have revealed that the solid exhibits superficial acid-base properties, attributable to silanol and aluminolic groups. Furthermore, the solid presents a point of zero charge (PZC) of 6.9 ± 0.2. The kinetic tests showed that a minimum of 48 hours was required to achieve a stationary state between Y^{3+} ions in aqueous solution and the solid phase. The data collected indicate that the process can be described by a pseudo-second order model. The isotherm data are interpreted in accordance with the Langmuir or Freundlich model. From the data collected at different temperatures, it is possible to obtain thermodynamic parameters[2], including the Gibbs free energy change (ΔG^0), enthalpy change (ΔH^0), and entropy change (ΔS^0), as well as kinetic parameters, including the activation energy.

$NaClO_4$ (M)	pH	Temperature (K)	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/(K×mol))
0.01	7.0 ± 0.1	298	-4.6 ± 0.5	29 ± 5	75 ± 10
		323	-7.4 ± 0.5		
0.1		298	-2.3 ± 0.5	14 ± 5	35 ± 10
		323	-3.7 ± 0.5		

Keywords: *geopolymer, yttrium, kinetic, isotherm, adsorption.*

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Identification of Ligands targeting Gs protein by Ligand-based NMR spectroscopy

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Heterotrimeric G proteins regulate intracellular signaling by transmitting signals from G protein-coupled receptors (GPCRs) located on the cell surface to cytoplasmic effector proteins, making them key therapeutic targets for a variety of diseases. The adenosine A2A receptor (A2AR), a member of the GPCR family, is involved in numerous physiological processes and is a key target in drug development. Targeting A2A and, simultaneously, its coupled Gs protein is a fashinating although challenging goal which could provide novel modulators of the this receptor. As starting point of this project, we are developing novel ligands of the Gs protein. Instead of using the heterotrimeric G protein which require low yeld expression in eukaryotic system, an engineered minimal G protein (mini-G) has been used. Mini-G, which was recently designed is composed solely of the GTPase domain of the stimulatory G protein Gs, and forms stable complexes with A2A receptor. Unlike, heterotrimeric G proteins, mini-G is highly expressed in E. coli, enabling a high expression rate and the production of significant quantities of protein. Using the established protocol, we have obtained large amount of mini-G and used it to test a series of rationally designed ligands using ligand-based NMR techniques such as STD and WL.

Keywords: *A2A receptor, GPCR, Gs protein, ligand-based NMR*

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Pharmacophore-based Inverse Virtual Screening and its application in accelerating the target identification and repositioning of bioactive compounds

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3D structure-based pharmacophores improved the evaluation of ligand/protein complexes in routine virtual screening procedures. The procedure is not time-consuming if a single target but, if a high number of proteins (e.g., belonging to the same pathological pathway) is considered, the time/benefit ratio is still unaffordable due to the complexity of the process. This bottleneck is why we created PharmaCore[4], an entirely automated process that generates 3D structure-based pharmacophores for proteins that fulfill particular criteria. The procedure requires the availability of experimentally determined protein/ligand complexes of the chosen target in the Protein Data Bank, which will be used to determine the correct orientation of the molecules inside the binding cavity. The collected structures are clustered according to the portion of the protein sequence they cover and superposed to the one at the best resolution. After that, following a recursive procedure, the pharmacophore hypotheses are generated for a chosen target, and, exploiting the fastness of the workflow, a whole panel of protein-related pharmacophores can be built. These hypotheses can be applied to a new kind of experiment: Pharmacophore-based Inverse Virtual Screening (P-IVS), to accelerate the target identification of bioactive compounds. This new method starts with an extensive molecular docking campaign between the desired compound(s) and the chosen reference structure of the panel. Subsequently, the quality of the produced protein/ligand complexes is quickly and accurately evaluated by rigidly screening them against the target-specific 3D structure-based pharmacophores. The identification and repositioning of AM879, a known ATAD2 inhibitor, on two unprecedented targets (BRPF1 and BAZ2B) is a successful example of P-IVS. In detail, AM879 was already known to interact with ATAD2, a bromodomain-containing protein, but not with BRD2, BRD3, and BRD4, belonging to the same family. Therefore, we used PharmaCore to develop pharmacophores for all the bromodomain-containing proteins for which more than four protein/ligand complexes were deposited in the Protein Data Bank, resulting in more than 1000 hypotheses for 16 targets. After that, AM879 was docked against the reference structure of each target, and the resulting poses were screened using the corresponding pharmacophores without altering their orientation. In this way, we considered both the chemical moieties of the compound and how they were arranged inside the target binding pocket. Interestingly, BRPF1 and BAZ2B emerged as promising targets despite no data being reported in the literature, and the binding with AM879 was confirmed by AlphaScreen assays. This new method represents, therefore, a valuable improvement in the target identification process, leading to more accurate and reliable results to guide the subsequent biological evaluations.

Keywords: *target identification, pharmacophore, drug repositioning.*

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Understanding the PED/PEA15-Phospholipase D1 interaction mechanism in type II diabetes through NMR spectroscopy

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PED/PEA15 (Phosphoprotein enriched in diabetes/Phosphoprotein enriched in the astrocytes 15 protein) is a cytosolic protein involved in various protein-protein interactions and thereby it modulates many cellular functions including apoptosis, proliferation and glucose metabolism. [1] In particular, PED (overexpressed in some forms of type II diabetes) binds to phospholipase D isoform 1 (PLD1) influencing both glucose-stimulated insulin secretion and insulin-stimulated glucose transport. [2, 3] The modulation of the PED/PLD1 interaction, indicating PED as a pharmacological target, represents the starting point for the development of new drugs able to inhibit this binding.
In this study, a selection and identification of PED ligands were performed through the NMR screening of a library of small molecules. The NMR characterization of PED/PLD1 interaction in cellular lysates is reported, also in presence of BPH03 (diphenyl scaffold), the best selected ligand. Our findings provide detailed information on the structural determinants of the PED/PLD1 interaction and indicate BPH03 as a valuable scaffold for production of novel compounds capable of modulate the binding, with possible therapeutic applications in type II diabetes. [4] New NMR data, consistent with the in-silico results, suggest that BPH03 disrupts the PED/PLD1 interface, displacing PLD1 from its interaction with PED. Moreover, computational methods were used to confirm previous results, revealing the existence of concealed, druggable pocket within PED-PLD1 binding surface. [5]

Keywords: *Proteins, small molecules, solution NMR, drug discovery*
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Metabolic Profiling of Plasma Distinguishes Indolent from High-Grade IPMNs using GC-MS

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Intraductal Papillary Mucinous Neoplasms (IPMNs) are pancreatic cystic lesions that produce thick mucus and have the potential to progress to invasive cancer. Originating from the epithelium of the pancreatic duct, IPMNs are classified into two categories: indolent and high-grade. Indolent IPMNs progress slowly and have a lower risk of developing into invasive cancer, whereas high-grade IPMNs are more aggressive and carry a significantly higher risk of malignancy. Differentiating between these forms early and accurately is crucial for effective patient management and treatment decisions.
Metabolomics, the comprehensive study of small molecules within cells, tissues, or biofluids, has emerged as a valuable approach for understanding disease mechanisms and identifying potential biomarkers. Gas Chromatography-Mass Spectrometry (GC-MS) is a robust and sensitive analytical technique widely used in metabolomics due to its ability to separate and identify volatile compounds with high precision.
Here, GC-MS was mainly used to perform an untargeted analysis of the metabolic profiles of plasma samples from patients with indolent and high-grade IPMNs: a total of 126 samples were analyzed, and chemometric analyses were employed to identify several metabolites that differentiate between the two IPMN categories. Resulting data were pre-processed and analyzed using advanced statistical and bioinformatics tools to identify pathways and metabolites that are significantly different between indolent and high-grade IPMNs.
Notably, glycolic acid, glucose, and gluconic acid were found to be particularly significant in distinguishing between indolent and high-grade IPMNs. These metabolites have also been observed in other studies, underscoring their potential relevance in IPMN diagnostics (2). Furthermore, our study identified several novel metabolites that have not been previously associated with IPMN pathology. Additionally, metabolites implicated in the Warburg effect, a phenomenon recognized for its association with cancer events, displayed notable differences (3). These findings underscore the relevance of metabolic dysregulation in distinguishing high-grade IPMN lesions, emphasizing their heightened resemblance to cancerous conditions. Such insights offer valuable implications for understanding the underlying mechanisms driving IPMN progression and hold promise for refining diagnostic and therapeutic approaches aimed at effectively managing this disease.

Keywords: *Intraductal Papillary Mucinous Neoplasms, IPMN, metabolomics, GC-MS, cancer*
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Cationic Azobenzenes as Light-Responsive Crosslinkers for Alginate-Based Supramolecular Hydrogels

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Azobenzene photoswitches are fundamental components in contemporary approaches aimed at light-driven control of intelligent materials. Significant endeavors are directed towards enhancing the light-triggered reactivity of azobenzenes for such applications and obtaining water-soluble molecules able to act as crosslinkers in a hydrogel. Here, we report the rational design and the synthesis of azobenzene/alginate photoresponsive hydrogels endowed with fast reversible sol–gel transition. We started with the synthesis of three cationic azobenzenes (AZOs A, B, and C) and then incorporated them in sodium alginate (SA) to obtain photoresponsive supramolecular hydrogels (SMHGs). The photoresponsive properties of the azobenzenes were investigated by UV–Vis and ^{1}H NMR spectroscopy. Upon irradiation with 365 nm UV light, the azobenzenes demonstrated efficient *trans*-to-*cis* isomerization, with complete isomerization occurring within seconds. The return to the *trans* form took several hours, with AZO C exhibiting the fastest return, possibly due to higher *trans* isomer stability. In the photoresponsive SMHGs, the minimum gelation concentration (MGC) of azobenzenes was determined for different compositions, indicating that small amounts of azobenzenes could induce gel formation, particularly in 5 wt% SA. Upon exposure to 365 nm UV light, the SMHGs exhibited reversible gel–sol transitions, underscoring their photoresponsive nature. This research offers valuable insights into the synthesis and photoresponsive properties of cationic, water-soluble azobenzenes, as well as their potential application in the development of photoresponsive hydrogels.

Keywords: *Ionic Azobenzenes, Photoisomerization, Supramolecular hydrogels, Sodium alginate, Crosslinkers*

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Innovative ultra-sensitive detection of neurodegenerative biomarkers by the pyro-electrohydrodynamic jetting

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Brain located protein biomarkers are generally recognized as the main cause of neurodegeneration mechanisms and hence as potential targets. Unfortunately, these proteins are present in low abundant concentrations in peripheral body fluids making difficult to provide an early diagnosis. Herein, we show the possibility to detect low abundant proteins as Tau or β-amyloid in urine samples at sub-picogram level, through the concentration effect of the pyro-electrohydrodynamic (p-jet) technique. An immunofluorescence protocol is then applied to concentrated p-jet spots for a quantitative analysis achieving very competitive limit of detection (LOD), thanks the possibility by p-jet to reduce drastically the diffusion effects in the antibody-antigen reaction. Moreover, our methodology is also validated by conventional spectroscopic techniques as circular dichroism (CD), Dynamic Light scattering (DLS), Infrared spectroscopy (IR) and fluorescence. Our aim is to test our technique into a compact version in clinical practice routine.

The antimicrobial peptide Temporin-L induces vesicle formation and reduces the virulence in S. aureus

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Most AMPs active against Gram-positive or Gram-negative bacteria exert their mechanism of action by directly interacting with cell membrane and cell wall, disrupting the lipid bilayer and leading to bacterial death. However, recent studies revealed that AMPs can also interfere with cellular processes and metabolic function by targeting intracellular molecules including nucleic acids and proteins [1].
The amphibian Temporins constitute a well-known family of AMPs with high antibacterial properties against Gram-positive and Gram-negative bacteria [2].
In this study, we evaluated the *in vivo* effect of Temp-L on methicillin-resistant *Staphylococcus aureus (MRSA)* by performing morphological studies using Transmission Electron Microscopy (TEM) that revealed the occurrence of several membrane protrusions from the cell surface, suggesting the formation of vesicle-like structures.
The vesicle-like structure was confirmed by Dynamic Light Scattering (DLS) showing a decrease in bacterial cell size and the formation of a second population whose size could be associated with extracellular vesicles. The global effect of Temp-L on *S. aureus* was deeply investigated by differential proteomics leading to the identification of up-regulated proteins involved in the synthesis of the cell membrane and fatty acids and down-regulated virulence factors. GC-MS analysis suggested a possible protective response mechanism implemented by the bacterium after treatment with Temp-L, as the synthesis of fatty acids was increased. Adhesion and invasion assays on eukaryotic cells confirmed a reduced virulence of *S. aureus* following treatment with Temp-L. These results suggested targeting virulence factors as a new strategy to replace traditional antimicrobial agents that can be used to treat infections, especially infections caused by the resistant pathogen *S. aureus*.

Keywords: *AntiMicrobial Peptides, drug resistance, morphological studies, differential proteomics, cell invasion*

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Marine Strategy Framework Directive: nutrient analysis in seawater samples

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The marine environment is a valuable fortune that requires protection, preservation, and safeguarding to maintain its biodiversity, vitality, and productivity. In response to the increasing need to mitigate anthropogenic pressures on marine waters, the European Parliament and the Council of the European Union issued the Marine Strategy Framework Directive (MSFD-2008/56/EC) on June 17, 2008 [1]. Italy transposed this directive through Legislative Decree No. 190 of October 13, 2010 [2], which was subsequently supplemented. The directive has a dual objective: safeguarding the marine ecosystem and ensuring the sustainability of human activities related to the sea. Objective assessment is carried out through an integrated study of 11 qualitative descriptors linked to environmental targets. Among all the descriptors, the focus is on Descriptor 5 whose objective is the request for minimizing human-caused eutrophication with particular regard to its negative effects on biodiversity and ecosystem degradation. Eutrophication, caused by the enrichment of nutrients, especially nitrogen and phosphorus compounds and silica, leads to negative outcomes such as biodiversity loss, ecosystem degradation, increased algal blooms, and oxygen deficiency in bottom waters, which alter benthic communities and generally degrade water quality. To monitor pollution, physical, chemical, and nutrient variables are assessed. Nutrient monitoring requires seawater sampling at various depths and distances from the coast according to reference module: for the M1 module are monitored nitrite, nitrate, ammonia, total nitrogen, orthophosphate, total phosphorus, and silicates in the water column; for 6A the input in nutrients (total nitrogen and total phosphorus) in areas where there are aquaculture facilities is investigated; for 6F ammonia, total phosphorus and total nitrogen are quantified. Samples for the analysis of nitrite, ammonia, orthophosphates and silicates are filtered, while for the analysis of total nitrogen and total phosphorus the sample is analyzed as is, but after having undergoing oxidative digestion in an autoclave. The analysis methods involve spectrophotometric measurement based on colorimetric reaction at different wavelengths depending on the analyte to be quantified, using APAT IRSA methods [3]. An EasyChem Plus discrete analyzer is used for this purpose. In general, for the three-year period 2021-2023, nutrient concentrations are low and in line with the trend of previous years with some nutrient peaks (ammonia, nitrate, total nitrogen and silica), in some months of the year and at some stations, correlated with river flows and thus rainfall.

Keywords: *MSFD, Descriptor 5, nutrient analysis, seawater*

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Phytochemical analysis of essential oil and methanolic extract from leaves of wild *Origanum vulgare L.* from central Italy, *in vitro* antioxidant activity and on HepG2 cell line

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Origanum vulgare L. is an important aromatic plant used since ancient times in folk medicine. Its importance in the scientific world is due to its special chemical composition made of bioactive compounds including polyphenols (phenolic acids and flavonoids) and compounds of a volatile nature (essential oil), which together make oregano endowed with multiple biological activities[1].
In this study, the volatile components of the essential oil extract (EO) and the polyphenolic components of the methanolic extract (ME) of leaves of wild *Origanum vulgare* from central Italy were identified by GC-MS and UHPLC-DAD respectively. The most abundant EO component was carvacrol, while there was a strong presence of rosmarinic acid in the ME. It is known that both components possess potential antioxidant power, as shown by *in vitro* assays (DPPH, ABTS and FRAP methods) carried out on both extracts. In addition, oregano ME and EO were added *in vitro* using human hepatoblastoma cell line HepG2 to assess possible biological activities for survival and oxidative stress. The results showed that, at the doses used, both the ME and the EO did not show obvious toxicity, as suggested by the unaltered proliferation rate of HepG2 cells. However, the ME at low doses (50 and 100 μg/ml) and the EO (0,005%), administered as a pre-treatment, showed a protective effect against oxidative stress, as inferred from the reduction in levels of 8-OHdG, a marker of oxidative damage to nucleic acids.

Keywords: *Origanum vulgare L.,* phytochemical analysis, essential oil, oxidative damage.

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HNBR crystallization behavior under stretching: comparison between a semi-crystalline and an amorphous samples

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A study of the structure-properties relationships of hydrogenated butadiene/acrylonitrile rubbers (HNBR) [1,2] is reported, focusing on the influence of the presence of initial crystals on the molecular chain orientation, and possible crystallization of amorphous chains by effect of stretching. The analysis is focused on an amorphous sample with 44 wt% of ACN (HNBR44) and an initially crystalline sample with 50 wt% of ACN (HNBR50). The samples have been provided by Arlanxeo company. It is shown that the amorphous sample HNBR44 experiences strain induced crystallization (SIC). The deformations marking the crystallization onset during stretching and the complete melting of the crystals during the release of the tension are 200 % and 100 %, respectively. The crystallinity index during the unloading step is greater than that occurring in the loading step due to “supercooling” (hysteresis). During stretching, the orientation of the amorphous chains increases linearly until the deformation at SIC onset is reached, and then reaches a plateau at greater deformations. Well oriented crystals are instead formed by effect of SIC. At variance with the amorphous sample, the initially crystalline sample HNBR50 shows a gradual increase of crystallinity during stretching with no hysteresis during the unloading and the loading steps. This behavior is ascribed to the nucleation effect exerted by pristine crystals. The orientation of amorphous chains increases linearly and does not reach a plateau, while the orientation of the crystals shows a more gradual increase compared with the orientation of the crystals formed by SIC. The study points out for the first time to the effect of presence of pristine crystals in HNBR samples.

Keywords: *HNBR, crystallization during stretching, chain orientation*

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Continuous-flow electrochemical oxidation of nitrogen compounds in livestock manure with an innovative reactor: The RiduciN Project

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The global livestock industry annually generates vast volumes of wastewater, posing a significant challenge for farmers in managing effluents from confined animal feeding operations. Livestock wastewater (LW) is typically rich in nitrogen compounds and other pollutants, posing risks to both human health and ecosystems if released untreated into water bodies. In southern Italy's Campania Region, nearly ten thousand livestock farming operations harbor around two hundred thousand cattle, leading to substantial nitrogen water pollution and vulnerability to contamination. The Council Directive 91/676/EEC (Nitrates Directive) imposes a maximum nitrogen pollution limit from livestock effluents in vulnerable zones at 170 kg of N per hectare per year, underscoring the urgent need for innovative and effective treatments to mitigate nitrogen compounds in water1.
In this context, the RiduciN project2, supported by the Campania Region, aims to address this critical issue by advancing scientific knowledge in chemical, environmental, and engineering fields. It focuses on pioneering treatment methods for zootechnical effluents, aiming to experiment, develop, and implement cutting-edge environmental technologies. Central to the project is the development of a pilot plant tailored for treating liquid waste from livestock. This plant utilizes natural zeolite adsorption and electro-oxidation techniques to enhance treatment processes. LW samples were collected from a farm in the Campania Region (specifically, the province of Caserta) for experimental laboratory testing. These tests involved the utilization of an innovative continuous-flow electrochemical reactor for the treatment of total nitrogen (TN) through electro-oxidation (EO) (Fig. 1). The reactor (volume of 500 mL) was characterized by dimensions of 63 cm in length, 6 cm in width, and 5 cm in height. It featured two mesh electrodes, each 1.0 mm thick and measuring 11 cm in height and length, with diamond-shaped openings of approximately 1 × 3 mm. These electrodes were separated by a 4 mm rubber gasket. The cathode utilized metal mixed oxide (MMO) Ti/IrO2-Ta2O5, while the anode employed Magnéli-phase titanium suboxides (TinO2n-1). To ensure a consistent flow, a fluid pump was employed, while a BPS-305 current power supply (Lavolta, London, UK) provided the necessary electricity directly to the electrodes. pH sensors were installed at both the inlet and outlet of the reactor to continuously monitor pH levels. Additionally, an industrial chiller maintained a constant temperature within the reactor.
Initial sample analysis revealed that the raw LW contained approximately 500 mg/L of TN and 446.9 mg/L of chloride, highlighting the necessity for effective removal treatments prior to discharge into water bodies. Figure 2 illustrates the preliminary findings from EO experiments conducted by varying the applied current density (J = 11.1 – 33.3 A m-2). It can be seen that increasing J from 11.1 to 22.2 A m-2 resulted in an enhancement of total nitrogen (TN) degradation efficiency from 21 to 26% after 180 minutes of treatment. This outcome can be attributed to the accelerated production of chlorine reactive species (CRS) on the anode surface, such Cl2, HClO, and ClO-, which play a crucial role in oxidizing the nitrogen species present in the system3. However, further elevating the current density to 33.3 A m-2 did not yield a significant improvement in system degradation performance, achieving only 28% of TN degradation. Increasing J theoretically results in a higher production of CRS, thereby accelerating the oxidation reaction and improving treatment removal efficiencies. However, it also entails a decrease in both the selectivity and the current efficiency of the system due to higher J leading to increased undesired side reactions, such as oxygen evolution and the generation of by-products (chlorate and perchlorate). This reduction in selectivity and efficiency ultimately diminishes the removal efficiency.
The preliminary lab activities showed promising result in removing nitrogen compound from complex livestock wastewater by using an innovative continuous-flow reactor. Over the next few months, the RiduciN project will persist in conducting additional experimental investigations to refine operational conditions and attain higher TN degradation efficiencies. The ultimate goal remains to develop efficient treatments for livestock LW, thus facilitating the implementation of good circular economy practices geared towards restoring and reusing water in agriculture.

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A promising class of SARS-CoV-2 M^{pro} inhibitors: Spiropyrrolidinone-based compounds

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During the COVID-19 period, the scientific community extensively worked to identify novel, effective SARS-CoV-2 inhibitors to fight the catastrophic pandemic. The drug discovery approaches focused on development of new direct-antiviral agents (DAAs) targeting essential proteins in viral life cycle such as main protease (M^{pro}) and Polymerase (RdRp).[1,2] In this context, our laboratory immediately started working on a wide project on CoV-M^{pro} inhibitors, mainly focused on design and synthesis of new peptidomimetic SARS-CoV-2 M^{pro} inhibitors. In the meanwhile, Pfizer disclosed, in April 2021, the discovery of Nirmatrelvir, a covalent reversible peptidomimetic inhibitor. Nirmatrelvir was the first in class M^{pro} inhibitor approved by the FDA in combination with the metabolic booster Ritonavir (Paxlovid).[3] Consequently, our research capitalized on the Nirmatrelvir structure in order to identify a new chemical entity sharing the peptidomimetic nature and potentially the same mode of inhibition. Therefore, we designed and synthesized several sets of analogues, incorporating a novel P1 moiety based on a spiropyrrolidinone structure to replace the γ-lactam ring of Nirmatrelvir, along with various amino acid substitutions in the P2-P3 sequence. The spiropyrrolidinone P1 residue, characterized by specific chirality of two stereocenters, perfectly fits into S1 pocket similarly to the classical γ-lactam ring. It retains the hydrogen bonds network with the side chains of His163 and Glu166 residues in S1 pocket and additionally interacts with the backbone of Phe140 within the S1 the binding site.
F2F-2020381 derivative exhibited an IC_{50} = 40 nM similar to the Nirmatrelvir potency, this compound confirmed the high quality of the design and it was the "front runner" of the series, In conclusion, we have identified a promising new analogue based on a distinct P1 residue instead of the typical γ-lactam ring found in the reference compound Nirmatrelvir. These preliminary but encouraging findings pave the way for an extensive structure-activity relationship (SAR) studies of this novel class of peptidomimetic M^{pro} inhibitors, aiming to enhance the potency both in enzymatic and antiviral cell-based assays.

Keywords: SARS-Cov-2 M^{pro} inhibitors, new P1mimetic, covalent reversible peptidomimetic.

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Binding of $[V_4O_{12}]^{4-}$ and Unprecedented $[V_{20}O_{54}(NO_3)]^{n-}$ to Lysozyme

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Polyoxidovanadates (POVs) constitute a sub-class of polyoxidometalates (POMs) with intriguing physico-chemical, electronic, catalytic, magnetic and, particularly, medical properties.[1] The mechanisms at the basis of POVs biological activity are not completely clear. However, there is no doubt that their binding to proteins plays a crucial role.[2] Recently, high-resolution crystal structures of lysozyme in the presence of the potential drug $V^{IV}O$(acetylacetonato)$_2$ under two different experimental conditions have been solved. The crystallographic study reveals the loss of the ligands, the oxidation of V^{IV} to V^{V} and the subsequent formation of adducts of the protein with two different polyoxidovanadates: $[V_4O_{12}]^{4-}$, which interacts with lysozyme non-covalently, and the unprecedented $[V_{20}O_{54}(NO_3)]^{n-}$, which is covalently bound to the side chain of an aspartate residue of symmetry related molecules.[3] $[V_4O_{12}]^{4-}$ has a cyclic structure with V^{V} in a tetrahedral geometry and held in its position by a network of hydrogen bonds. $[V_{20}O_{54}(NO_3)]^{n-}$ can be described as an unusual octadecavanadate (V_{18}) cage of composition $[V_{18}O_{46}(NO_3)]^{n-}$ attached to two additional V atoms, each bound to five oxygens in a VO_5 group where one O atom is shared with the cage; this latter group is in its turn coordinated to the side chain of Asp87 and Asp87* of a symmetry mate. This POV is reconstituted by its symmetry mate and the occupancy is equal to 0.60.
Thus, an unusual example of protein structure in the presence of an unprecedented V oxide polyhedron and a new arrangement of the $[V_{18}O_{46}(NO_3)]^{n-}$ cage, as well as its interaction with a protein, have been reported for the first time. The results show that protein crystals can serve as chemical reaction vessels and that soaking of metal compounds within protein crystals coupled to the determination of the X-ray structure is a strategy to capture unexpected metal compound structures, like POVs formed from solutions containing different V species. Data also suggest that protein crystals can be used to stabilize elusive POVs, providing nice examples of stabilization of unrevealed POV structures by formation of electrostatic interactions and H-bonds within the protein crystal packing. These results provide new structural basis for interpreting experiments carried out with the potential drug $V^{IV}O(acac)_2$ and similar $V^{IV}O$-based drugs and for understanding their significant biological properties.

Keywords: *vanadium-based drugs, polyoxidovanadates, polyhedral, V–protein interaction*

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Synthesis and interactome characterization of a novel cytotoxic quinazolinone library

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Quinazolinones are the oxidized form of quinazolines, nitrogen-containing heterocyclic compounds that consist of a benzene ring fused with a pyrimidine ring.[1]
In recent years, many quinazolinones have been acknowledged for their striking polypharmacological framework, making them an intriguing scaffold for the development of new therapeutic candidates for a wide range of biological effects, such as antitumor, antimicrobial, anti-inflammatory, anticonvulsant, antiviral, antidiabetic and insecticidal activities.[2,3,4] In the pharmaceutical field, quinazolinones are the building blocks of more than 150 naturally occurring alkaloids isolated from different plants, microorganisms, and animals. Scientists maintain a continuous interest in this moiety due to their stability and relatively easy methods for preparation. Modifications on quinazolinone system changed significantly their biological activity due to changes in their physicochemical properties. Structure–activity relationship (SAR) studies of quinazolinones revealed that positions 2, 6, and 8 of the ring system are significant for different pharmacological activities.
Starting from this panorama, the focus of our research project was the synthesis and the interactome characterization of a series of novel bioactive quinazolinone derivatives, endowed with cytotoxic activity on MCF7 and DU-145 cancer cells. Starting from a small set of synthesized quinazolinones, a multi-disciplinary strategy has been carried out on the most bioactive compound to disclose its potential biological targets by functional proteomics, using a label-free mass spectrometry-based platform coupling Drug Affinity Responsive Target Stability (DARTS) and targeted Limited Proteolysis- Multiple Reaction Monitoring (t-LiP MRM).

Keywords: 4-(3H)-quinazolinones; Structure activity relationship; Heterocyclic scaffold; Functional proteomics; Protein-ligand interaction

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Structural Insights into the Agonist Mechanism of Action of Tetracyclic Antidepressants on Serotonin Receptors

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Serotonin (5-hydroxytryptamine, 5-HT) is a key neurotransmitter that plays a crucial role in regulating various physiological processes through its interaction with serotonin receptors (5-HTRs), part of the family of class A G protein-coupled receptors. [1] Among the 13 known 5-HTR subtypes, 5-HT_{1e}R remains understudied, despite its therapeutic potential. [2] Through cryo-electron microscopy, mutagenesis and computational studies, we elucidated mianserin and setiptiline's unique agonist-like binding poses at 5-HT1eR, challenging their classification as pan-antagonists at 5-HTRs. [3] A detailed structural analysis shows these compounds interacting uniquely with 5-HT receptors, inducing agonist binding and cellular response. In particular, mianserin and setiptiline exhibit specific binding to the receptors, activating signaling pathways within the cell that promote a positive biological response. This agonist mechanism is further confirmed through mutagenesis studies showing that specific mutations do not affect the compounds' affinity, suggesting that the overall binding mode and allosteric networks play a key role in drug efficacy. Computational analyses further supported the notion that the specific binding poses of these drugs, coupled with receptor-specific allosteric mechanisms in 5-HT_{1e}R and 5-HT_{1F}R, contribute to their agonist activity. The *in silico* approach employed in this study, which included Molecular Dynamics (MD) simulations, structural interaction fingerprint (SIFt) analysis and transfer entropy calculations, provided a comprehensive overview of the molecular mechanisms underlying the agonist mechanism of action of tetracyclic compounds. MD simulations highlight how specific interactions between ligands and receptors influence the conformation and the allosteric communication between the orthosteric ligand binding site and the receptor–G protein by analyzing the contributions of residues to the allosteric communication between the orthosteric ligand binding site and the receptor–G protein interface via transfer entropy analysis of our MD simulations, and, thus, offering valuable insights for the design of new compounds with optimized pharmacological profiles. By uncovering the molecular basis of the agonist activity of tetracyclic antidepressants at these receptors, this research opens new avenues for drug development and the design of more effective treatments for neurological and psychiatric disorders.

Keywords: *serotonin receptors, tetracyclic antidepressant, computational studies, anti-migrain*
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POPs determination in serum and semen of contaminated areas of northern Italy

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Contamination caused by anthropogenic activity is nowadays a field of increasing interest since old and new emerging pollutants, acting as endocrine disruptors, are responsible of many disorders such as cancers, neurological, metabolic and reproductive. This work aims at the determination of organic contaminants, persistently disseminated in the environment and in organisms and their relationship with the increasing infertility. Specifically, 478 serum and 456 semen samples were analyzed for the determination of ipa, pcb, dioxins bisphenols and metals. Samples were collected in northern Italy areas, in order to monitor the Ambiental contamination due to Miteni's activities. Metal analysis was performed by ICP-MS that allowed to examine 27 metals in traces and ultra traces, for each sample. ICP-MS investigation showed the presence of high levels of chromium and nickel in serum samples, while rubidium, strontium, lead and Barium were founded in semen. Quantitative analysis of IPA, PCB, Dioxins was performed by Tandem Mass Spectrometry in Multiple Reaction Monitoring, exploiting the high sensitivity, rapidity and selectivity of this technique. IPA and Bisphenol resulted to accumulate more in semen than in the serum while PCB were in higher concentrations in semen.

Keywords: *POPs, endocrine disruptors, infertility, tandem mass spectrometry*

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Kinetic investigation of Silybin A/B chemo-enzymatic acetylation in a flow milli-reactor

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The separation of silybin A (SilA) and B (SilB) diastereomers in optically pure compounds is challenging due to their very similar physical and chemical properties. However, such separation is crucial for evaluating the biological activity of diastereomers SilA and SilB, which show very different performance in pharmacological applications like treating prostate cancer, liver diseases, and Alzheimer's disease [1]. The most common isolation method is based on high performance liquid chromatography, but it is slow and with a yield in pure SilB of hundreds of milligrams per day being unsuitable for a possible scale-up. An alternative chemo-enzymatic separation method, utilizing immobilized lipase CALB catalyst to stereoselectively acetylate silybin B [2], offers advantages in terms of higher productivity, selectivity, and scalability, particularly when applied in flow reactors.
This research explores the kinetics of SilA/B acetylation catalyzed by Novozym 435 in a continuous flow milli-reactor, analyzing various temperatures, volumetric flow rates, and initial Sil A/B concentrations. The novelty of this work is underscored by the lack of prior kinetic studies on this reaction. Using an axial dispersion model and assuming an Eley-Rideal mechanism, mass balance equations for the packed bed reactor were derived and solved by using MATLAB R2022b as calculation software. Figure 1 compares the simulated and experimental data for the three investigated variables. The examined reaction showed a null apparent activation energy, explaining the temperature insensitivity of the final acetylated Sil B concentration. Additionally, increasing volumetric flow rates led to decreased steady-state concentrations of acetylated products, signifying a kinetic regime reaction. A maximum initial silibinin concentration was identified, beyond which conversion did not improve.

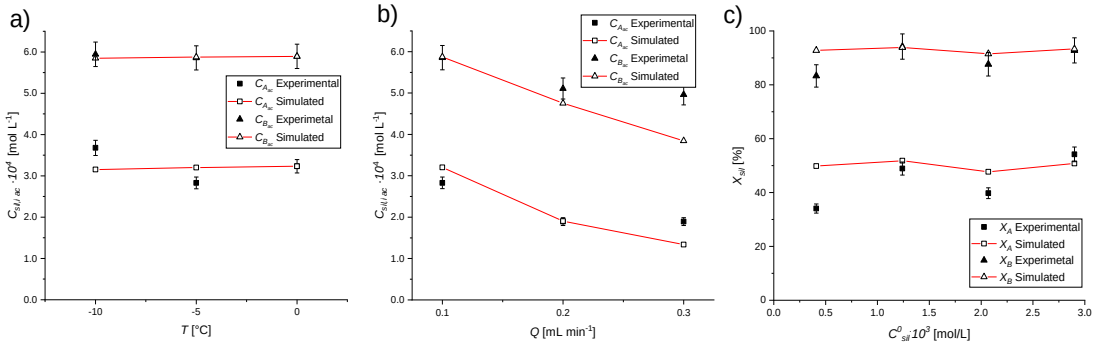

Figure 1- Comparison between experimental data (black symbols) and simulated data (white symbols) at: a) different temperatures *T*, b) different volumetric flow rates *Q*, and c) different initial concentrations C^0_{sil}.

Keywords: silybin; chemo-enzymatic resolution; lipase; acetylation reaction; flow-chemistry.

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Bioactive peptides hidden in human endopeptidases: the case of three novel cryptides identified in metalloproteinase 19 by a computational-experimental platform.

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Several peptides produced during maturation processes of precursor proteins play central roles in innate defence systems in all complex life forms [1, 2]. We recently identified three human antimicrobial cryptides in metalloproteinase 19 (MMP-19) by a computational-experimental platform [3]. The three putative antimicrobial peptides (AMPs) were named r(P)YLL19, r(P)YLL33, and r(P)PRT33, since they correspond to regions 1-19, 1-33, and 247-279 of MMP-19 precursor protein, respectively. P refers to the presence of a Pro residue at the N-terminus of recombinant peptides obtained upon acidic hydrolysis of an Asp-Pro labile peptide bond located between the sequences of the AMP of interest and of the carrier protein onconase. The three peptides were recombinantly produced by using a cost-effective production procedure previously set-up by our research group [4]. Recombinant AMPs were found to be endowed with a broad-spectrum anti-microbial activity, being active against both Gram-negative and Gram-positive strains comprising also clinically isolated and antibiotic resistant bacterial strains. MMP-19-derived AMPs were also found to be endowed with anti-biofilm properties being able to affect all the three main stages of biofilm development, i.e., attachment, formation, and detachment, as indicated by crystal violet and confocal laser scanning microscopy analyses. Furthermore, no toxic effects were detected when peptides were tested on eukaryotic cell lines. Starting from these promising results, MMP-19-peptides have been selected as suitable bio-agents to be loaded into nanoparticle (NPs) obtained from bacterial cellulose (BC), with the aim to set-up a system to efficiently deliver AMPs of interest for applications in biomedical, food or cosmeceutical fields.

Keywords: *antimicrobial peptides, cryptides, antibiofilm, nanoparticles*

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Possibilities and perspectives in the hybridization of metal organic frameworks (MOFs) with non-conventional graphene related material

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The production of hybrids is an attractive approach to enhance metal organic framework (MOF) properties, since the combination of MOFs with suitable materials can improve the overall functionality, porosity, and thermal/magnetic/electric properties to meet specific requirements. Polymers, metal oxides, nanoparticles, carbon nanotubes, quantum dots, and graphene related materials (GRMs) are the most common materials used for the preparation of MOF composites and hybrids [1].
The integration of MOFs and functional materials as graphene related materials (GRM) is gaining a fast-growing positioning both in consolidated topics (gas storage and separation, hydrocarbons adsorption, catalysis and photocatalysis, electrochemical applications) and in emerging fields (electrochemical sensors, microwave adsorption, energy harvesting) [1-4]. Thanks to the presence of heteroatoms containing functional groups and the aromatic sp^2 domains, GRMs can act not only as fillers, but also participate in bonding interactions, enhancing the coordination bonding and influence the growth of MOF. Depending on the structure of the MOF, the interactions between metal nodes and the GRM can lead to an increase of the defect and micropore volume of the composites, resulting in a higher concentration of unsaturated metal centers that serve as adsorption sites for gases. The intercalation with GRM also provides for electrical conductivity, while the pristine MOF is usual insulating [5]. Anyway, the fundamental understanding of the MOF-based hybrid formation has been very limited.
In this contribute we proposed an overview on the synthesis, the structural properties and the gas adsorption capacities of hybrid materials obtained intercalating the structure of HKUST-1, a benchmark copper-based MOF, with GRMs derived from the chemical destructuration of a nanostructured carbon black [6]. Hybrids differing for the content or the type of GRMs have been synthetized and structurally characterized by conventional (SEM, TG, XRD, FTIR, N_2 adsorption) and advanced techniques (ssNMR, NEXAFS and XANES). In addition, also the conductive properties and the adsorption capacities towards CO_2, CH_4 and H_2 of the hybrids have been investigated.

Keywords: *metal organic frameworks, graphene related materials, hybrid materials, adsorption tests*

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Identification of new c-Myc binders through a combined *in silico* and STD/NMR-based approach

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c-Myc is a transcriptional factor located in the nucleus and encoded by c-MYC gene belonging to the Myc proto-oncogene family. It is involved in different cellular processes, e.g., cell growth, differentiation, metabolism, and death. Dysregulation in expression level and activity of c-Myc is related to the occurrence and progression of various cancer types, thus highlighting c-Myc as one of the most significant human oncogenes. With the aim of identifying new potent c-Myc inhibitors, a workflow based on computational studies combined with the Saturation Transfer Difference (STD)/NMR investigations led to the definition of rationale to target this oncoprotein.[1] Firstly, in order to face the problem of the absence of co-crystal structures of the protein with known binder, *in silico* molecular docking experiments were carried out to reproduce the binding pose of the known c-Myc inhibitor 10058-F4 towards the c-Myc binding domain recognized,[2] supported by STD/NMR experiments. Employing the available crystal structure of c-Myc complexed with its protein partner Max, thus accounting for the bioactive conformation of c-Myc in the heterodimer arrangement, docking calculations of 10058-F4[2] were performed in order to obtain the best pose to adopt as a reference for the evaluation of commercially available compounds in a virtual screening campaign. Afterward, ~200,000 commercially available items were subjected to molecular docking experiments and, finally, 20 compounds were selected for the subsequent biophysical evaluation step. In this study, STD/NMR, a useful technique for understanding ligand binding sites on proteins and for providing intricate details of the proximity of the ligand to protein residues, was successfully applied for c-Myc for the first time, thus representing a breakthrough for investigating this intrinsically disordered protein (IDP). Among the first set of tested compounds, one item showed promising binding towards c-Myc in STD/NMR experiments, further corroborated by *in vitro* experiments. These findings, obtained through the applied workflow based on *in silico* and spectroscopic approaches, are crucial since they shed light on the ligand-protein interactions of c-Myc, of which there are only a few known binders. More importantly, these outcomes also serve as a key basis for the development and discovery of novel c-Myc inhibitors, thus offering a strong starting point for the challenging investigation of this intrinsically disordered protein.

Keywords: *c-Myc, molecular docking, STD/NMR, cancer, drug discovery*

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Polyethylene and polysiloxane persisting on surface seawater and in wastewater treatment plants: recovery, ponderal quantification, microstructural analyses and origin investigation

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Microplastics and, more in general, synthetic polymer debrides (SPD) of anthropic origin are accumulating in the environment, in particular in marine water [1] and sediments, with risks associated for aquatic organisms and humans not well wholly understood. In this contribution it is reported a new method for the isolation of microplastics from surface seawater and wastewater treatment plants, and the quantification by solution ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) with respect to a known concentration of an internal standard (mesitylene) dissolving the microplastics in 1,1,2,2-tetrachloroethane-d_2 (TCE-d_2). TCE-d_2 is a high boiling solvent that allowed the analysis and quantification of poly(ethylene) (PE) and poly(dimethyl siloxane) (PDMS) MPs at 80 °C. These polymers are thus persisting on seawater because of their low density and the ponderal concentrations were quantified in mg/m^3. This method was used in an actual case study in which 120 surface seawater samples were collected during two sampling campaigns in the Mediterranean Sea (from the Gulf of Salerno to the Gulf of Policastro in South Italy). The developed analytical protocol allowed for achieving unprecedented simplicity, rapidity and sensitivity. The ^{1}H and ^{13}C NMR structural analysis of the PE debris indicates the presence of oxidised polymer chains with very low molecular weights. The analytical protocol has also been applied to a real case study (50 samples) from two campaigns of sampling in WWTPs located at Tavernola (Battipaglia (SA), Italy) and Punta Gradelle (Vico Equense, (NA), Italy) where different approaches to the WW treatment are used, namely the bed adhered biomass (MBBR) and membrane bioreactor (MBR) technologies [2], respectively.

Keywords: *microplastics; nanoplastics; recovery; seawater; marine; quantification, microfiltration, NMR spectroscopy, standard method*

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Mimics of glutathione peroxidase: selenoglycoconjugates

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This work is part of a project focused on the synthesis of mimics of glutathione peroxidase (GPx), an enzymatic antioxidant characterized by the presence of selenocysteines in the active site. In the last 20 years the exploitation of antioxidant activity of selenobased-compounds has improved, just due to the use of Ebselen, a selenocompound, as GPx mimic.[1] However, their clinical use seems to be compromised by the low solubility in water.[2] For this reason is difficult to achieve optimal therapeutic doses in the blood. In order to deal with this problem is possible to take advantage of the pharmacological properties of selenium with the chemical-physical properties of sugar. To this aim in the last decade the research is focused on the use of selenosugars. In this frame, in attempt to optimize the antioxidant properties of selenosugars, the glycoconjugation with a polyphenolic unit, which is a molecule capable of inhibiting or disabling the action of free radicals, could be considered. Polyphenols are, indeed, a class of compounds with antioxidant and antiviral properties which are related to the number of the phenolic ring, the position of the hydroxyl groups and the number of unsaturation of the molecule. The purpose of this work is the synthesis of selenoglycoconjugates able to exploit in a synergic way the antioxidant propriety of selenium with those of chelators and antioxidant of polyphenols, to prevent an oxidative stress state.
Based on previous results obtained on D-ribose,[3] we, in turn, employed as starting sugar the D-mannose to synthesize the selenosugar and it was then conjugated, thanks to the reactivity of primary hydroxyl, to a polyphenolic unit.

Keywords: *selenosugar, polyphenol, selenoglycoconjugate*

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Exploring the interaction between DNA G-quadruplexes and an RG-rich peptide

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G-quadruplexes (G4s) are non-canonical secondary structures formed within DNA or RNA G-rich sequences and characterized by the stacking of two or more G-tetrads.[1] G4 structures have attracted great attention since the discovery of their formation in a cellular context. G4s are particularly enriched in cancer-related genes and regions predisposed to cancer amplification, representing promising targets for therapeutic intervention.[1] The formation of G4 structures is strictly controlled through several proteins that bind and stabilize or unfold them. In this context, a database (G4IPDB) containing more than 200 G4-binding proteins from various organisms has been created.[2]
A recent study revealed a common motif among 77 human G4-binding proteins, consisting of a 20-mer R/G-rich sequence, named NIQI (Novel Interesting Quadruplex Interaction motif).[2] However, its ability to physically interact with G4 structures has never been experimentally demonstrated.
The interaction between the RG/RGG-rich NIQI peptide and various G4 DNA structures was explored for the first time using several biophysical techniques.[3] In particular, Circular dichroism (CD) experiments were performed to evaluate the peptide's ability to thermally stabilize the G4s. Microscale thermophoresis (MST) and Isothermal titration calorimetry (ITC) experiments were carried out to quantify the binding affinity and stoichiometry for NIQI interaction with the various G4s, and to verify its selectivity for G4s over duplex DNA. Moreover, NMR spectra were recorded to understand the binding mode of NIQI, and the DNA regions affected by peptide binding. Finally, by adopting the alanine scanning approach, we systematically evaluated the impact of each arginine and serine residue on the peptide binding to different G4s, thus identifying the key and less relevant amino acids in the interaction with these DNA G4s. Overall, our experimental evidence revealed that NIQI strongly binds to G4s and exhibits good selectivity for this structure over the canonical duplex DNA, laying the groundwork for new peptide-based G-quadruplex ligands, promising alternatives for interfering in DNA-protein interactions and potentially offering therapeutic avenues.

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

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Stereoselective Polymerization of 1- Vinylcyclohexene and (*S*)-4-Isopropenyl-1-Vinyl-1-Cyclohexene and their Copolymerization with Styrene and Terpenes

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The shift towards environmental sustainability is driving the use of renewable resources in industrial processes, including polymer production. Significant progress has been made by using renewable monomers to produce polyolefins, moving away from traditional petrochemical materials. This change addresses concerns about limited fossil fuel resources. A significant advancement in synthesizing polyolefins from renewable monomers involves using metal complexes with OSSO-type ligands, which show high catalytic activity and selectivity not only in polymerizing bio-based monomers, leading to efficient and high-quality polyolefin production[1,2]. This study evaluates the catalytic performance and stereoselectivity of two titanium complexes with OSSO-type ligands in the polymerization of a bio-based monomer, obtaining a high regio- and stereoregular polymers. We achieved the polymerization of (*S*)-4-Isopropenyl-1-vinyl-1-cyclohexene (IVC), a naturally derived monomer synthesized from perillaldehyde, which is most abundantly found in the herb perilla[3]. Previously, a similar study was conducted using 1-vinylcyclohexene (VCH) as a model monomer because its structure is identical to IVC except for the isopropenyl substituent. In addition, the copolymerization of these monomers with styrene and two terpenes, β-myrcene and β-ocimene, was also accomplished confirming not only the extreme versatility of the [OSSO]-type titanium complexes in the copolymerization reactions but also enlarging the repertoire of more sustainable polymeric materials for practical applications.

Keywords: *Stereoregular polymerisation, [OSSO]-type titanium complexes, terpene-based monomers, catalyst performance, isotactic polymers*

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Discovery Of A New Potent And Selective Histone Deacetylase 6 Inhibitor In Triple Negative Breast Cancer Treatment

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

Keywords: *epigenetics, HDAC6, breast cancer*

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Amino acid quantification by mass spectrometry without derivatization to characterize campanian cheeses.

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Amino acids (AA) are important metabolites for the metabolism, growth, maintenance and repair of tissues. Moreover, AAs also serve as precursors for many hormones, neurotransmitters [1], and other specialized metabolites [2, 3].
The dosage of the amino acids in foods is a very common practice due to the biological relevance that these molecules have in human health and many studies attest to how the amino acid component is responsible for the organoleptic properties of food products [4-7].
Most AAs are small aliphatic molecules incapable of fluorescence or UV absorption, making the AA analysis a difficult task [8]. Classical analytical methods for the analysis of AAs involve their chemical derivatization [9, 10] and subsequent detection by using different approaches such as HPLC-DAD or more sensitive and specific technique such as LC-MS/MS or GC-MS. Therefore, the derivation reagents must be carefully selected, ensuring rapid reactions that lead to the formation of stable products for a long time.
The present work showed the development of a method for the identification and quantification of the 20 proteinogenic AAs by mass spectrometry in multiple reaction monitoring (MRM/MS) ion mode. The proposed case study concerned the application of the method developed on a cohort of samples of cheeses from Campania, produced from milk of different animal origins (*Bos taurus*, *Capra hircus*, *Ovis aries*). The cheese made by a specific species was also produced by leaving each form covered by a layer of grape on the surface to give the cheeses a peculiar flavor and aroma. Therefore, the current study proposed to compare the AA profile between the outer and the inner cheese form for both type of cheese (with or without grapes).
During the ripening process, proteolysis is considered the most important biochemical process that occurs in hard and semi-hard cheeses, as it promotes a series of reactions that determine the sensory characteristics of the cheese [11]. During this process, the cheese protein matrix is decomposed due to the action of proteinases and peptidases from different sources: lactic ferments, coagulation enzymes, microbial enzymes from the starter culture and secondary microbiota, and other exogenous enzymes.[12]. This phenomenon influences the texture, sensory perception, and overall quality of the cheese [12, 13] and implies the liberation of peptides that can have biological activity (such as casomorphins)[14, 15], the increase in the content of free amino acids and other compounds volatile and non-volatile compounds, such as esters, ketones or aldehydes [12, 16].
This approach guarantees a simple and fast method for the absolute quantification of AA in several cheese samples performed by a highly selective and sensitive method based on chemical fingerprinting of the target molecules.

Keywords: *amino acids, mass spectrometry, Multiple Reaction Monitoring, food characterization*

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$In_2O_3@TiO_2/Cu_2O$ for H2 development by solar energy

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Hydrogen (H_2) represents one of the most promising energy resources to replace fossil fuels due to its high energy density, which can be exploited without releasing emissions into the environment.[1] Water splitting (H_2O) through solar energy is one of the most studied methods to produce H_2 because it is a green reaction that requires low energy and does not form secondary products.[2] For this reason, scientific research is highly focused on developing increasingly efficient photocatalysts, with titanium dioxide (TiO_2) still being extensively investigated due to its properties (high chemical stability, low cost, and non-toxicity). However, the use of TiO_2 as a photocatalyst is affected by some issues such as low solar light utilization efficiency and rapid recombination of photogenerated carriers. One of the strategies to address these drawbacks, besides the use of sacrificial agents, is to combine this oxide with other semiconductors to improve its photocatalytic performance.[3] Despite being a semiconductor that can well adapt to TiO_2, non-toxic and cheaper compared to other metals, indium has been poorly investigated in the H_2O splitting reaction, and most of the catalysts reported in the literature based on In_2O_3/TiO_2 have been tested in other types of photocatalytic reactions.[4] In this work, a new $In_2O_3@TiO_2$-based catalyst was developed and tested in the H_2O splitting reaction. Additionally, to improve photocatalytic activity, the binary compound was loaded with cuprous oxide nanoparticles (Cu_2O NPs), often coupled with TiO_2 due to their absorption in the visible region.[5] Satisfactorily, the new ternary photocatalyst $In_2O_3@TiO_2/Cu_2O$ yielded an excellent H_2 production of 9.6 mmol h^{-1} g^{-1}, which is 48 times the amount produced by TiO2 alone, proving to be more active compared to other In2O3-based catalysts reported in the literature.[6]

Keywords: *hydrogen, solar energy, photocatalysis*

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Novel 1,3,4-Oxadiazoles as Potent PD-L1 Antagonists: A One-Pot Synthetic Approach for Enhanced Immune Checkpoint Inhibition

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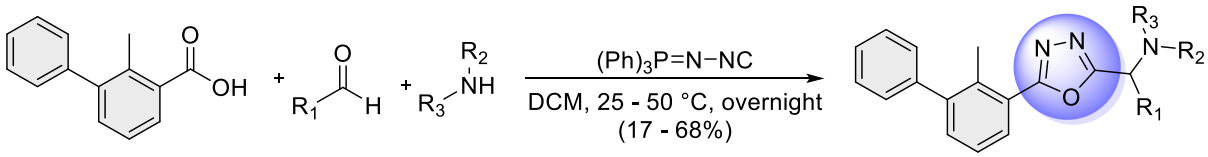
Cancer immunotherapy targeting PD-L1 and the PD-1/PD-L1 pathway offers a promising approach in the treatment of cancer pathology. PD-L1 interaction with the PD-1 receptor on T cells represents one of the immune checkpoint blockade (ICB) strategies that help cancer cells in evading immune detection. In addition, its overexpression is often related to poor prognosis in various type of cancer. Immune checkpoint inhibitors (ICIs) working as cancer immunotherapy drugs are able to interact with one of these two key players, blocking their interaction and unleashing the immune system.
Despite the success of antibody-based therapies, they come with many limitations and side effects. Thus, small-molecule inhibitors targeting PD-L1 can represent an attractive alternative.



Previous research suggested that biphenyl core can represent an election moiety for targeting PD-L1.[1,2] Building on this, we here present the synthesis and evaluation of a new set of compounds containing the biphenyl core combined with the 1,3,4-oxadiazole moiety, as new promising PD-L1 antagonists.
The selected synthetic approach consists of a novel and efficient one-pot multicomponent procedure, involving an Ugi-4CR/aza-Wittig synthesis conducted under mild conditions. In this case the biphenyl synthon works as acid component in the Ugi reaction, together with the appropriate amines and aldehydes, using (N-isocyanimino)triphenylphosphorane (NIITP) as the isocyanide component and key player in the Wittig cyclization.[3] Taking advantage of this procedure we have been able to synthesize a small set of compound, which tested by HTRF showed promising binding properties.

Keywords: *immune, anticancer, PD-L1, multicomponent, oxadiazoles*

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Paper-based assay utilizing sequence-specific dye for the colorimetric detection of TATA box

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TATA box is a DNA sequence found within many eukaryotic promoters for protein-coding genes. It consists of alternating adenine/thymine (A/T) pairs, i.e. TATAT, and is known as a transcription start site region. The TATA box-binding protein (TBP) is essential for the transcription of ribosomal, messenger and transfer RNAs by the three eukaryotic RNA polymerases: Pol I, Pol II, and Pol III. Abnormalities in TBP have been linked to several diseases, such as incurable neurodegenerative disease, and gastric cancer.[1,2] Recent strategies for identifying sequences with alternating A/T, such as the TATA box, have utilized small organic cyanine dyes. These cyanine dyes can spontaneously assemble in DNA duplexes and have shown sequence-dependent behavior. For instance, DISC2(5), a member of the cyanine dye family, is exploited for the detection and quantification of amplified nucleic acids in colorimetric assays[3] and in agarose gel electrophoresis[4]. These methods, however, require amplification steps of the target sequences, costly equipment, and highly qualified personnel, which impacts their sustainability and limits their use in decentralized areas. In the context of providing novel solutions, we developed for the first time a paper-based colorimetric assay for the detection of TATA BOX. The portable all-in-one device comprises a 3D-printed dark chamber, which serves to capture images of the paper-based assay followed by image processing via the free ImageJ tool. The portable device utilizes a simple smartphone as a detector and includes a slider mechanism to facilitate the deposition of the paper-based substrate containing the sample. Light office paper is used as the paper-based substrate and is pretreated with wax to create hydrophobic barriers that enables sample drying. A small sample volume of 8 µL is dried on the paper substrate and the DNA duplexes are detected by the addition of the DISC2(5) dye. The dye assembles within the double-stranded DNA targets, resulting in the appearance of a blue color, also visible by naked eye, whose intensity increases with higher target concentration present in the sample. After optimization studies of dye concentration, target concentration and various paper-based substrates, a calibration graph was obtained for TATA sequences of two different sizes, ranging from 0 to 100 µM. Among these preliminary results specificity studies were also performed with sequences consisting of different amount and positions of A/T, i.e. random and non-alternating A/T pairs. These studies showed promising results, indicating the potential for applying our proposed device to real sample applications.

Keywords: *paper-based, dye, colorimetry, TATA, smartphone*

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Development of peptide-based molecular strategies to interfere with protein misfolding and aggregation of prion

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Prions are responsible for fatal neurodegenerative disorders known as prion diseases or transmissible spongiform encephalopathies (TSEs). The key molecular event in the pathogenesis of prion diseases is the conformational conversion of the cellular prion protein (PrP^{C}) into its misfolded counterpart, scrapie prion (PrP^{Sc}), which initiates a cascade of neurodegenerative events [1]. Currently, there is no effective treatment for prion diseases that underscore the urgent need for continued research into therapeutic interventions for this persistent threat to public health. Recently, we identified the structural and dynamical determinants controlling the prion misfolding process by which PrP^{C} (HuPrP90-231) converts to an amyloid fibril through the formation of a β-sheet-enriched intermediate state (β-PrPI) involved in the initial stages of prion fibrillation.
Moreover, our study demonstrated the importance of the coupling, through transient electrostatic interactions, between the N- and C-terminal domains of PrP^{C} in modulating long-range microsecond-millisecond conformational dynamics, which in turn regulate the folding process and prevent the formation of β-PrPI and prion aggregation [2]. In this study, taking advantage of our findings, we intend to target the prion pathogenic conversion by developing peptide-based strategies able to interfere with the initial stages of prion misfolding and aggregation process avoiding the formation of stable intermediate states and/or oligomeric species involved in the amyloid assembly mechanism. Here, we report the investigation of the conformational features and the evaluation of the binding interactions with HuPrP (90-231) of a twenty-one amino acid peptide, encompassing the region from Lys^{23} to Ser^{43} of the N-terminal domain of Human prion (HuPrP), called MANTRAP 1. NMR data indicate that MANTRAP 1, displaying a high degree of conformational flexibility without adopting any preferential secondary structure, is able to transiently interact with HuPrP (90-231).

Keywords: *Prion disease, Cellular prion protein, β-PrPI, MANTRAP 1*

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Optimized Extraction of Phenolics and Procyanidins from Seven Medicinal Herbs for Nutraceutical Development

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The main objective of the study was the optimization of the procyanidin (PC) solid/solvent extraction process, using grape pomace, to ultimately determine the PC profile and bioactivity of seven medicinal herbs to potentially use in nutraceutical products. The highest PC yields were achieved by the optimized extraction conditions which were evaluated singularly and validated by the valuation of % recovery and % matrix effect. The HPLC-FLD method of quantification of monomeric to trimeric PCs was validated by the ICH guidelines[1]. The proposed quantification method was applied to the seven medical herbs. Quantification of 17 polyphenols has been performed by MS working in Multiple Reaction Monitoring (MRM) using a 4000 Qtrap spectrometer. HPLC-HESI-MS/MS analysis performed on the herbal extracts led to the qualitative identification of 27 further polyphenols. Pearson correlation analysis (PCA) results exhibited higher Pearson coefficient (R2) and significance (p-value) values between total flavonoid content and the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) scavenging activity results, indicating flavonoids as the most relevant active substances of the extracts. Overall, *Paullinia cupana* Kunth. seed extract contained the highest level of PCs among all the extracts evaluated and had an antioxidant activity of 693.63 ± 48.04 µmol Trolox equivalent/g of dry weight, thus, it was the matrix that would serve better as the source containing a vast range of flavonoid-type antioxidant agents to be used in prospective nutraceutical products. It would also reduce inflammation rates by interfering with oxidative stress signaling and suppressing the pro-inflammatory signaling transductions, effective in the prevention of chronic inflammation and metabolic disorders[2].

Keywords: *procyanidin; medicinal herbs; extraction; HPLC-HESI-MS/MS analysis; antioxidant*

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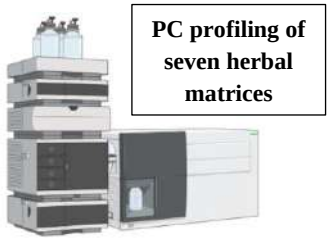

***Hamamelis virginiana* L.**

***Sambucus nigra* L.**

***Eleutherococcus senticosus* Maxim.**

***Paullinia cupana* Kunth.**

***Sambucus nigra* L.**

***Peumus boldus* Molina**

***Crataegus monogyna* Jacq.**

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Enhancing Sensitivity and Robustness in Untargeted Metabolomics: Microbore UHPLC-HRMS Approach

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Untargeted metabolomics is invaluable for understanding disease mechanisms and treatment responses [1]. While narrowbore LC-HRMS (2.1 mm I.D. columns) is common, higher sensitivity is needed for low-volume samples. Microbore columns (1.0 mm I.D.) offer potential but are underutilized in metabolomics [2]. We developed a sensitive microbore UHPLC-HRMS method for metabolomics, maintaining robustness, demonstrated using human plasma, Dried Blood Spots (DBS) and cells samples.

Methods

All the analyses were performed on a nano/microflow Vanquish Neo UHPLC coupled to a Orbitrap Exploris 120 mass spectrometer. Both RPLC and HILIC chromatography were performed with sub-2 micrometer fully porous particles 1.0 mm I.D. columns. UPCLC-HRMS analyses were performed in data dependent acquisition (DDA), using a resolution of 60000 in MS1 and 15000 in MS2, both positive and negative electrospray ionization were employed. Human plasma, DBS and cells samples were used as test samples. Data alignement, filtering and metabolite annotation was performed with Compound Discoverer 3.0. Comparative analyses on 2.1 mm ID column were performed on a Vanquish Flex system coupled to a Orbitrap Exploris 120 MS. **Preliminary Data**

Flow rate, gradient, injection volume, column temperature and source parameters were investigated by a mixture of metabolites with different retention and ionization behavior. A flow rate of 100 µL/min resulted to be the best condition among those tested. Microbore UHPLC–HRMS shows excellent reproducibility of chromatographic retention time (≤0.5% coefficient of variation, CV) and peak area (≤2.3% CV), together with sharp and symmetrical peaks with an average Full Width at Half Maximum (FWHM) of 0.05 min. Additionally, the microbore approach showed higher sensitivity in full scan (FS)-DDA, resulting in LOD and LOQ values of 0.95 and 3.18 ng/mL. Compared to narrowbore method, a ~2-fold increase in response was obtained on the same sample, this turns in almost twice MS1 spectral features and metabolite annotations at MSI level 2. The system robustness was proved in 48 hours and more than 300 consecutive injections. Notably, a reduction of solvent consumption is also obtained (300 mL vs 1.5L), making this approach more environmentally sustainable. As proof of concept, the microbore LC-HRMS method was tested in the profiling of CRC organoids metabolome, uncovering modulation in several metabolic pathways following chemotherapy.

Keywords: *microbore, untargeted metabolomics, sensitivity*

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Transition metal complexes *versus* amyloid aggregation: new potential therapeutic strategies in neurodegeneration.

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Metallodrugs have attracted considerable interest in the field of neurological disorders due to their potential to effectively modulate the aggregation of amyloid proteins, which is a hallmark feature of several neurodegenerative diseases [1]. These conditions are characterized by the accumulation of misfolded proteins, such as amyloid-beta (Aβ) in Alzheimer's disease or alpha-synuclein (αSyn) in Parkinson's disease, and the subsequent formation of amyloid plaques [2]. Transition metal complexes, due to their unique properties, are able to interact with amyloid proteins through coordination, oxidation or hydrolysis mechanisms and inhibit or enhance the formation of large oligomers. Herein, we report on the ability of several metal complexes to modulate the amyloid aggregation of model amyloid systems, focusing in particular on several ferrocene-based and paddlewheel diruthenium (Ru_2^{5+}) complexes [3-4]. By means of a multi-technique approach including various spectroscopic (e.g., fluorescence assays, UV-vis absorption, and electrospray ionization mass spectrometry) and microscopic (scanning electron microscopy and confocal microscopy) techniques and cellular assays, their Mechanisms Of Action (MOAs) have been elucidated. Overall data can open new perspectives for the application of metal-based agents as potential new therapeutic drugs in neurodegeneration.

Keywords: *metallodrugs, paddlewheel diruthenium complexes, neurodegenerative disorders*

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Simulation of organic mixed ionic and electronic conductors with a combined classical and quantum mechanical model

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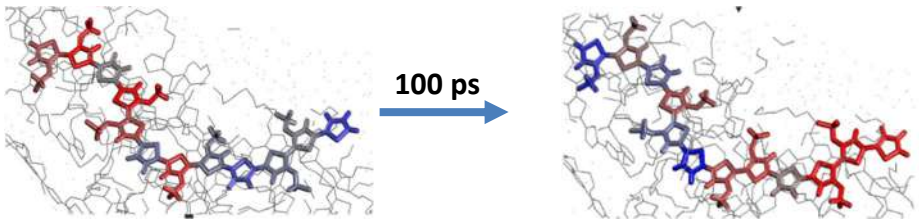
Organic materials that efficiently couple electronic and ionic charge transport (OMIEC) have been recognized as essential in a wide range of technologies [1], from energy storage and generation [2] to nanomedicine and healthcare [3], thanks to their ease of processing, flexibility, low cost, and because they can be finely tuned, e.g. to ensure perfect integration with cellular tissues for nanomedicine or a light weight for energy storage.

Theoretical predictions could represent a great help in developing new materials, tailored for any given application. However, they face the fundamental obstacle that, in these systems, the excess charge is very mobile, and the dynamics of the polymer chain cannot be accurately described with a model including only fixed point charges. Ions and polymer are comparatively slower and a methodology to capture the correlated motions of excess charge and ions is currently unavailable. Considering a prototypical interface for an archetypal OMIEC (poly-thiophene with glycol side chains), we constructed a scheme based on the combination of MD and QM/MM to evaluate the classical dynamics of polymer, water and ions, while allowing the excess charge of the polymer chains to rearrange following the external electrostatic potential [4]. We find that the location of the excess charge varies substantially between chains. The excess charge changes across multiple timescales, as a result of fast structural fluctuations and slow rearrangement of the polymeric chains. Our results indicate that such effects are likely important to describe the phenomenology of OMIEC, and we are working on the introduction of additional features in the model to enable the study of processes such as electrochemical doping.



Pictorial representation of charge redistributions in an OMIEC during the dynamics

Keywords: *Organic mixed ionic electronic conductors; Molecular Dynamics; QM/MM*

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Unveiling the competitive diffusion of binary gas mixtures in polymers: the case of carbon dioxide and alkanes in nanoporous-crystalline polyphenylene oxide

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Natural gas and petroleum gas (ethane, propane, buthane) are energy carriers more sustainable than coal. Moreover, natural gases like propane are seen as green refrigerants which will soon replace highly polluting hydrofluorocarbons (HFCs). Purification of fossil fuel gases may be accomplished with polymeric membranes. In this work, we investigated the sorption of binary gas mixtures constituted of carbon dioxide and either methane, ethane or propane in Poly(2,6-dimethyl-1,4-phenylene)oxide (PPO) endowed with nanoporous crystalline domains [1]. This material is a good candidate for fuel gas sweetening due to its peculiar pore morphology and distribution. The experiments were conducted at sub-atmospheric pressures and in the range [24, 35] °C to prevent any structural modification induced by the sorbates.
We resorted to a hyphenated technique coupling in situ FTIR Spectroscopy and Barometry [2]. First, we conducted pure gas sorption tests. At thermodynamic equilibrium, the IR signal of each low molecular weight species was isolated in the polymer phase and its absorptivity was calibrated with Barometry [1]. Then, binary sorption tests were conducted and the solubility of each species in PPO was measured at thermodynamic equilibrium and during diffusion with vibrational spectroscopy. Competitive sorption with ethane or propane produces a depletion of the solubility of carbon dioxide with respect to pure gas sorption at the same partial pressure. Moreover, at short times during co-diffusion, carbon dioxide reaches supraequilibrium loading. Such a phenomenon is described by an overshoot in the kinetics and is attributed to the solubility competition between the two penetrants. We effectively interpreted it with the Maxwell – Stefan theory of multicomponent diffusion coupled with the classical Langmuir's adsorption model and showed that the overshoot is a proof of uphill diffusion in PPO [3].
At the thermodynamic conditions investigated, the separation performances of PPO are analogous to DDR type zeolites despite the different chemical nature of the two adsorbents [4]. For the first time, the competitive diffusion of two gases in a polymer film is unveiled.

Keywords: *Poly(2,6-dimethyl-1,4-phenylene)oxide, Gas Mixtures, FTIR Spectroscopy, Competitive Sorption Dynamics*

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Globular proteins as suitable tool to obtain amyloid fibrils

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Amyloid fibrils are usually considered pathological aggregates responsible for several neurodegenerative human diseases.[1] At the same time, it is becoming clear that the capability of some small globular proteins to form ordered supramolecular structures can be exploited to obtain nanostructured materials. To neatly aggregate globular proteins need to unfold, at least partially.[2] Indeed, there is a strong correlation between a decreased conformational stability of the native state and an increased propensity to aggregate into amyloid-like structures. In fact, protein fibrillation is an intricate process influenced by different parameters, extrinsic to the protein, such as pH, temperature, ionic strength, and protein concentration, or intrinsic, like charge, hydrophobicity, patterns of polar and non-polar residues, and the propensity to adopt different secondary structure motifs.[3] Moreover, the advantage of fibrillar protein aggregates consists in the possibility to modulate their formation or their structural characteristics by changing these parameters or by designing mutants.
Among proteins with this ability, the derivative of the sweet plant protein monellin, called MNEI, is particularly appealing. Aiming to improve MNEI stability and aggregation propensity, different mutants have been designed by mutating single residues. Among these mutants, one in particular, bearing the most promising mutations, has been held up as a "super stable mutant", Mut9. The new protein showed high stability in acidic and neutral environments and a higher melting temperature than that of MNEI opening the possibility of exploring other experimental conditions to obtain fibrils. These features suggested a deep study of Mut9 aggregation tendency by a multimethod approach. In particular, the kinetics of aggregation and the parallel changes of the protein structure, under different experimental conditions, have been studied by ThT fluorescence binding assay and FTIR spectroscopy respectively, while fibrils shape and morphology have been investigated by TEM.[4] A macroscopic characterization by SEM has also been carried out thanks to fibrils huge dimensions.
Altogether these results support the increasing evidence that MNEI and its variants are suitable models for the production of protein aggregates useful as potential building blocks for the construction of nanomaterials for various technological and biological applications, such as drug delivery, cancer therapy, or environmental biosensing.

Keywords: *amyloid, globular proteins, protein fibrillation, nanostructured biomaterials*

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Marine Strategy Framework Directive: metal analysis in marine sediments

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Our seas are characterized by a great variety of habitats, in which there is a very rich marine biodiversity; they are a source of support for a multiplicity of services, from food supply to tourism activities, to the regulation of fragile ecosystem and climate balances. The need to reduce anthropogenic impacts on marine waters, and to preserve the ecological diversity and vitality of clean, healthy, and productive seas through the sustainable use of the marine environment, led the European Union to issue the Marine Strategy Framework Directive 2008/56/EC [1], transposed in Italy by the Legislative Decree No. 190 of October 13, 2010 [2]. The Directive, based on an integrated approach through the study of 11 descriptors, sets as a goal for member states the achievement of good environmental status for their marine waters and identifies, within each European marine region and based on hydrological, oceanographic and biogeographic factors, sub-regions within which member states must carry out monitoring and identify appropriate programs of measures. Among all of them, the focus is on Descriptor 8. The objective of this descriptor is to assess the concentration of contaminants in marine sediments; thus, they do not exhibit levels that give rise to pollution effects. The substances investigated include synthetic products such as pesticides and antifoulants, non-synthetic compounds such as heavy metals and hydrocarbons, and pharmaceuticals. Exiting official methods for the analytical determination of metals in marine sediments involve sample drying (at T = 30-35°C) followed by sieving (< 2mm). On the sieved fraction, pseudo-total digestion of the sediment is conducted following the EPA 3051A [3] or EPA 6020B [4] method, which involves mineralization with a strong acid mixture, in a closed microwave system. Instrumental determination of metal content follows, was conducted by inductively coupled plasma mass spectrometry (ICP-MS), still following EPA methods. The results did not show any exceeding of the levels set by legislation although high concentrations were detected for some elements. In particular, the high levels detected for arsenic can be attributed to the geogenic origin of this element, due to the volcanic systems active in coastal areas.

Keywords: *MSFD, metals analysis, Descriptor 8, marine sediments*

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Development of a HPLC-MS/MS method for the characterization and quantification of intact glucosinolates in *Catozza* rapeseeds

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Glucosinolates (GSLs) are anionic sulfur-containing secondary metabolites of the Brassicaceae family, chemically described as β-thioglucoside N-hydroxysulfate derivatives. The analysis of intact GSLs in food matrices has historically represented a challenge for their physicochemical properties and for the presence of the enzyme myrosinase. Myrosinase is stored separately in the near myrosin cells. After cell damages, myrosinase hydrolyzes GSLs in several toxic breakdown products (e.g., isothiocyanates) [1]. Therefore, the first assumption has been the assessment of the best myrosinase inactivation conditions to preserve intact GSLs content. Among all species, *Catozza* rapeseeds (Brassica rapa L. var. rapa DC.) were selected as food matrix for this study to re-evaluate the commercial potential of native ecotypes of Campania region. The optimization of matrix processing provided the best myrosinase inactivation conditions by a 30-minute heating treatment at 80 °C, leading to the highest GSLs content. Two extractions methods were performed on the rapeseed meal to obtain hydroalcoholic and food-grade aqueous *Catozza* rapeseeds extracts. The aqueous extraction method was validated performing the matrix effect and recovery assays. The quantification was performed by a targeted HPLC-MS/MS analysis in negative acquisition mode. The method was developed by using a mixture of 6 GSLs analytical standards. The method was validated according to the ICH validation guideline [2]. According to the low values obtained for both precision and accuracy by intraday and interday analysis, the developed HPLC-MS/MS method was considered a reliable protocol for the identification of 17 intact GSLs and the quantification of 6 intact GSLs [3]. This study represents the starting point for further in-depth investigation of *Catozza* rapeseeds use for GSLs-based formulations for nutraceuticals purpose.

Keywords: *Glucosinolates, Rapeseeds, Myrosinase, HPLC-MS/MS*

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Unlocking the potential of protein-derived peptides to target G-quadruplex DNA: From recognition to anticancer activity

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Noncanonical nucleic acid structures, particularly G-quadruplexes (G4s), have garnered significant attention as potential therapeutic targets in cancer treatment [1,2]. They can be formed by stacking of at least two G-tetrads, a planar association of four guanines, connected by intervening loops [1].
Here, the recognition of G4 DNA by peptides derived from the Rap1 protein is explored [3], with the aim of developing novel peptide-based G4 ligands with enhanced selectivity and anticancer activity [4]. Biophysical techniques were employed to assess the interaction of a peptide derived from the G4-binding domain of the protein with various biologically relevant G4 structures. Through alanine scanning mutagenesis, key amino acids crucial for G4 recognition were identified, leading to the discovery of two peptides with improved G4-binding properties. However, despite their *in vitro* efficacy, these peptides showed limited cell penetration and anticancer activity. To overcome this challenge, cell-penetrating peptide (CPP)-conjugated derivatives were designed, some of which exhibited significant cytotoxic effects on cancer cells. Interestingly, selected CPP-conjugated peptides exerted potent anticancer activity across various tumour types via a G4-dependent mechanism.
These findings underscore the potential of peptide-based G4 ligands in cancer therapy and pave the way for the development of novel therapeutic strategies targeting these DNA structures.

Keywords: *DNA G-quadruplexes, peptides, anticancer agents, biophysics*

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Synthesis and characterization of β-myrcene/styrene and β-ocimene/styrene copolymers

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The objective of this work is to study the structure-property relationships of two new classes of sustainable materials, obtained using monomers derived from biomass. Hybrid styrene-based copolymers with terpene molecules such as β-myrcene and β-ocimene, were studied. The copolymers have a blocky microstructure. Myrcene and ocimene are olefinic monomers obtained from biomass. They are combined with styrene, an aromatic monomer, to produce copolymers with a wide range of adjustable properties, depending on the composition and conditions of polymerization.
Samples with myrcene/styrene and ocimene/styrene ratios 30/70, 50/50, 70/30 and 80/20 mol/mol were synthesized using a titanium complex as catalyst activated with MAO. Polymerizations were carried out at 70 ºC in a nitrogen atmosphere. [1]
For both series the glass transition temperature (T_g) increases as the styrene content increases. Differential scanning calorimetry thermograms show relaxation phenomena close T_g, attributed to the presence of long styrene sequences, as highlighted by annealing experiments at different temperatures. All samples are amorphous. They show X-ray powder diffraction profiles characterized by a double halo typical of polystyrene, and remain amorphous even after annealing treatments.
Copolymers based on myrcene with styrene content greater than or equal to 30 mol% show a high rigidity and experience viscous flow upon stretching at 70°C. The sample with 20 mol% appears as a soft material, able to flow even at 40 °C.
The samples based on ocimene shows the same characteristics. Exceptions occur for the samples with 70 and 80 mol% of ocimene, which show double $T_{g,}$ due to phase separation of styrene rich and styrene poor domains.
From the SAXS analysis, recorded at different temperatures, the size ζ of heterogeneities at nanometric scale was estimated using the Debye-Bueche equation. The oscillations of the ζ parameter value are probably due to tendency of alike comonomers to form small aggregates, the size of which are subjected to changes depending on the comonomer content, the temperature, and the thermal history in general.
The present study has enabled the identification of new classes of terpene-based hybrid systems, with potential applications for surface coating and with properties that can be adjusted according to composition.

Keywords: *Sustainable Thermoplastics; Renewable and biodegradable polymers; Biomass polymers; Circular economy polymers; Biocompatible polymers.*

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Alkaline and acid Red mud-Metakaolin based geopolymers for adsorption of methylene blue

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Red mud (RM) is the primary waste product generated through the Bayer process for alumina production from caustic digestion of bauxite. It requires a proper management due to its high alkalinity and for this reason its disposal represents one of the biggest issues in the mining industry. New technologies are being developed for its treatment and reuse, according to circular economy and environmental protection. [1]
RM combined to metakaolin represents a suitable material for developing geopolymer formulations. Geopolymers are usually obtained from aluminosilicate precursors by polycondensation in an alkaline environment using sodium or potassium silicate. During this reaction, a three-dimensional network of SiO_4^{4-} and AlO_4^{5-} tetrahedra, with bridging oxygen atoms, is formed. Na^+ or K^+ from sodium or potassium silicate are used to balance the negative charges that are formed on the Al tetrahedral site. In addition to activation in an alkaline environment, in recent years an acid-type activation has also been developed regarding the geopolymerization reaction. As for the acid activating solution, a phosphoric acid solution is used. Acid activation allows the production of poly(silico-alumino-phosphate) characterized by the presence of $[AlO_4]^{5-}$, $[SiO_4]^{4-}$ and $[PO_4]^{3-}$ units. [2]
In recent years, the use of treated wastewater has been proposed by regulatory agencies to increase water supply and cope with water scarcity. Thus, the remediation of dye pollution, deriving mainly from the textile industry is of fundamental importance. [3] Geopolymers offer advantages over the reference activated carbon adsorbent, characterized by high energy consumption during the production process and demanding regeneration process. [4]
In this paper, we report the preparation of alkaline and acid geopolymers with the incorporation of high RM content (50 wt%) as raw material, in line with circular economy principles to be used as an adsorbent in solution. The adsorption capacity of pollutants was evaluated for the removal of methylene blue, also a well-known cationic dye commonly found in wastewater from textile industries.

Keywords: *geopolymer*, *red mud*, *methylene blue*

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Development of a screen-printed electrode for the detection of Alkaline phosphatase as biomarker for cancer diagnosis and monitoring

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Alkaline phosphatase (ALP) has been studied as a potential biomarker of several types of diseases. The enzyme is involved in several physiological processes, including bone development, liver function, and digestion. Elevated serum ALP levels have been associated with bone metastasis in several types of cancer, including prostate, breast, and lung cancer. Increased ALP activity is thought to contribute to the progression of these cancers by promoting cell growth, invasion, and metastasis; on the other hand, low ALP expression has been observed in some tumor types, such as breast cancer and colorectal cancer, and may be associated with reduced tumor cell differentiation and increased cancer cell proliferation [1]. To date, the determination of ALP in human serum is performed according to a standard colorimetric method based on the absorbance of the yellow-colored product formed during the reaction between the ALP enzyme and pNPP (p-nitrophenylphosphate), with limitations related to sample preparation, interference with other tests, and the need for careful interpretation of results [2]. For these reasons, a screen-printed electrode capable of monitoring diverse levels of ALP was developed. The performance of the developed biosensor was studied by cyclic voltammetry, chronoamperometry and differential pulse voltammetry in 50 mM Tris-HCl (pH=7) as a buffer solution [3]. After optimization of several parameters, such as the reaction time between the enzyme and the substrate and the optimal substrate concentration, the system was tested at increasing levels of ALP in buffer solution and in 20% serum, achieving good linearity and a detection limit of lower than 10 U/L for both, buffer and serum, confirming the possibility of monitoring ALP levels in clinical samples over a wide range of enzyme concentrations quickly and easily, using a small amount of sample. The implementation of this type of biosensor offers high sensitivity and selectivity, portability, ease of use and cost-effectiveness, making them suitable for POCT applications.

Keywords: *electrochemical biosensors, alkaline phosphatase, cancer biomarkers*

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Innovative Oxoindoline Glutamine Mimic: Design and Synthesis of Peptidomimetic Inhibitors for SARS-CoV-2 3CLpro

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Since 2019, the Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), alongside other emerging infectious diseases like Zika, Chikungunya, Dengue, poses a significant challenge for the scientific community in developing antiviral agents able to block, eradicate and prevent further the virus spread. The 3-chymotrypsin-like protease ($3CL^{pro}$), also known as the Main protease (M^{pro}), represents an attractive drug target due to its central role in viral replication and the conservation of its active site across CoVs family. This cysteine protease specifically recognizes the sequence Leu-Glu as the P2-P1 residues. The peptidomimetic inhibitors almost always requires P1glutamine mimic, the γ-lactam ring in the P1 side chain is shared by most potent SARS-CoV-2 $3CL^{pro}$ inhibitors discovered during last years [1]. The core of the project is focused on the design of new peptidomimetics with a reversible covalent warhead. The inhibitors were designed by incorporating a novel moiety, the oxoindoline as P1 residue, with different the warheads, and P2/P3 sequences of compounds already known in literature such as GC-376, MI30 and Nirmatrelvir [2]. Covalent docking calculations of F2F-2020101, selected as a representative analogue within this new class, suggested that the P1 oxoindoline forms hydrogen bonds with the side chains of Glu166 and His163, and with the backbone of Phe140, establishing the essential interactions typically found in the P1 glutamine mimic residues. The synthesis was performed via Boc-AA chemistry in solution, followed by a convergent synthetic approach, to obtain both the final di/tripeptides with aldehyde and nitrile warhead. The compounds were tested *in vitro* in the enzymatic and antiviral assays to determine their potency and broad-spectrum profile. In particular, F2F-2020101 and '232 resulted as the most promising compounds among all the derivatives, showed IC_{50} in the nM range against the SARS-CoV-2 and MERS $3CL^{pro}$ and an EC_{50} in μM in the antiviral cell-based assays. The data proved the quality of the design of the P1 residue, never reported before in inhibitors so far, and can be considered a starting point to discover new potent and broad-spectrum $3CL^{pro}$ inhibitors.

Keywords: *SARS-CoV-2, COVID-19, 3CLpro inhibitors, peptidomimetics, reversible covalent inhibitors*

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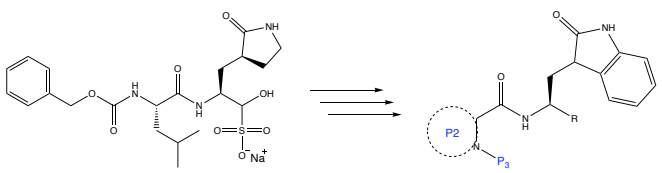

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Structural effects of the protein corona formed on different nanoparticles in the coelomic fluid of the sea urchin Paracentrotus lividus

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The identification of proteins present on the surface of nanoparticles (NPs), known as the protein corona, and the study of their structural variations are essential for clarifying the environmental safety of NPs and facilitating the regulation of their use [1]. NPs, with dimensions ranging from 1 to 100 nm, are widely used in personal care products, antibacterial preparations, and water remediation and are subsequently dispersed into the environment. The protein corona that forms on the surface of NPs upon contact with biological fluids plays a fundamental role in mitigating or stimulating ecotoxicological responses. At the molecular level, conformational changes occurring in the proteins within the corona upon interaction with NPs could affect their fate and biological outcomes [2]. To date, studies have primarily focused on the effects of NPs and the associated protein corona on humans, neglecting their effects on aquatic species, including the marine ones. Here we investigate how different NPs of various sizes and core composition, such as titanium dioxide (nTiO2), functionalized polystyrene (PS-NH2; PS-COOH), and silver (AgNPcitLcys) acquire different protein corona compositions upon incubation with the coelomic fluid (CF) of the Mediterranean Sea urchin, *Paracentrotus lividus*.
Preliminary TEM and SEM analyses confirmed the formation of a protein corona around the NPs, regardless of their chemical core composition. DLS parameters such as hydrodynamic diameter, polydispersity index and Z-potential revealed significant changes in NP's surface charges and size upon incubation in the sea urchin CF.
While some of the main components of the corona on PS-NPs had been previously identified through conventional MS analyses, in this project we used a shotgun proteomics approach to provide information on the nature of the CF proteins adhered to the different NPs. Briefly, protein fractions detached from NPs underwent enzymatic hydrolysis. The obtained peptides mixture were analysed by tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS). Data analysis and interpretation was carried out with various bioinformatics software for protein identification and quantification. Our preliminary results highlight a different protein composition in samples of CF incubated with the various NPs above described.

Keywords: *Nanoparticles, Coelomic fluid, proteomics, sea urchin.*
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Functional proteomics-aided interactome analysis of bioactive pyrazolyl-ureas

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Over the last twenty years, 5-pyrazolyl-ureas (5-PUs) poly-pharmacological profile has been deeply investigated, with a particular focus on their anti-angiogenic potential [1, 2]. In this scenario, a 5-PUs library has been synthesized in Genova University and, among such compounds, GeGe-3 and STIRUR-41 showed a remarkable anti-angiogenic profile: GeGe-3 blocked human umbilical vein endothelial cells (i.e., HUVEC) proliferation and migration and tumors pathological angiogenesis [3], whereas STIRUR-41 reduced the migration of a neuroblastoma cancer cell line (i.e., HTLA-230), counteracting its ability to form capillary-like structures [4]. Thus, to link GeGe-3 and STIRUR-41 biological profile to a suitable protein partner, their *interactome* has been deeply investigated, in HUVEC and HTLA-230 cells respectively, through *label-free* functional proteomics. Drug Affinity Responsive Target Stability (DARTS) [5] and targeted Limited Proteolysis-Multiple Reaction Monitoring Mass Spectrometry (t-LiP-MRM) [6] were indeed optimized and applied. These approaches share the principle that, due to the interaction with a molecule, a protein undergoes conformational changes that result in its lower sensitivity to limited proteolysis, when performed in native conditions. Hence, DARTS identified Calreticulin (i.e., CALR) and Ubiquitin Specific Protease 7 (i.e., USP-7) as GeGe-3 and STIRUR-41 most reliable interacting proteins in HUVEC and HTLA-230 cell lysates respectively, as further validated through Western Blotting, Then t-LiP-MRM data, which pinpointed CALR and USP-7 regions involved in the interaction with GeGe-3 and STIRUR-41, allowed us to draw a clearer picture of the biological consequences of such 5-PUs interaction with their partners, guiding the choice of specific *in vitro*/*in cell* assays [7, 8]. The interaction with CALR in its Ca^{2+}binding domains was indeed reflected in GeGe-3 ability to alter Ca^{2+}intracellular shift in HUVEC cells, modifying their cytoskeletal proteins organization, as demonstrated by *in cell* assays. Furthermore, STIRUR-41 interaction with USP-7 in its catalytic site explained its ability to inhibit USP-7 de-ubiquitination activity, as demonstrated by an *in vitro* assay. Thus, *label free* functional proteomics emerged as a powerful tool for an in-depth investigation of bioactive 5-PUs mechanism of action at a molecular level.

Keywords: *5-pyrazolyl-ureas, functional proteomics, mass spectrometry, limited proteolysis.*

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Nucleobase functionalized peptides: a new strategy for targeting ATP and GTP in cancer cells.

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Nucleopeptides represent a novel class of biomolecules where the peptide backbone incorporates nucleobases in the side chains (Fig. 1)1. The inherent versatility of nucleopeptides arises from the potential to manipulate both the peptide and nucleobase constituents, modulating favorable characteristics such as biostability, biocompatibility, multifunctionality, and the ability to spontaneously self-assemble in aqueous solutions2. Herein, we present an ongoing investigation into the US-assisted3 synthesis of tailored nucleopeptides aimed at identifying novel binders for ATP and GTP . While these nucleopeptides feature diverse amino acid sequences, they share distinctive functional moieties: a nucleobase-bearing amino acid (NBA) in the C-terminus (Lys) to bind Adenine or Guanine of the ATP or GTP, respectively; aromatic L-Phe and hydrophobic L-Leu, to enhance aggregation processes after target sequestration; three L-Arg residues to interact with the phosphate groups of ATP/GTP in cell and facilitate cell penetration through electrostatic interactions with negatively charged molecules on the cell surface; L-Ser as a hydrophilic spacer to balance the hydrophobic component and improve the solubility of the nucleopeptides in aqueous environments. Preliminary CD studies reveal nucleopeptide-ATP/GTP interactions by adopting distinct binding modes. NMR studies are in progress to gain further insights into their local conformational peculiarities and structural changes driving the target recognition process. Remarkably, some of these nucleopeptides, except for those bearing an acetyl group that replaced nucleobase on the side chain of Lys, also exhibited gel-forming abilities, highlighting the involvement of the nucleobase in sol-gel transition and opening new perspectives for drug delivery applications

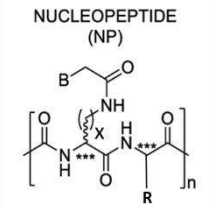

Figure 1: Nucleopeptide (NP) structure

Keywords: *nucleopeptide, ATP, GTP, targeting*

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Analyzing nicotine protection mechanism against amyloid toxicity by NMR-metabolomics: an exploratory study

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Pharmacometabolomics is emerging as an essential tool for analyzing the mode of action and toxicity mechanism of approved drugs and new molecular entities [1]. NMR showed great potential in this field since it is a non-destructive, highly reproducible, and quantitative method requiring relatively easy sample preparation [2].
Nicotine, the primary alkaloid synthesized from tobacco plants, is often blamed for its association with smoking and addiction. However, in the last decades, there has been a renewed interest in nicotine as a potential treatment option in neurodegenerative diseases, like Alzheimer's disease (AD), due to its anti-inflammatory, anti-apoptotic, pro-cognitive, and anti-protein aggregation effects. It is well known that nicotine stimulates the nicotinic acetylcholine receptor (nAChR), activating an anti-inflammatory pathway to reduce inflammatory responses, depression, attention, and cognitive deficits; furthermore, nicotine is a lipophilic agent and can penetrate the cells and acts independently on nAChR [3].
In addition, nicotine has been shown to slow down the aggregation of Aβ(1-42) in vitro by linking to the α-helix structure [4,5]. Given the complexity of nicotine action in AD, we performed a 1H-NMR metabolomics analysis on cell cultures treated with nicotine and amyloid recombinant protein to clarify nicotine's protective action in a simple model with an innovative method that, unlike others, may give a systemic explanation of nicotine anti-AD molecular mechanism.
The effect of nicotine was monitored in neuroblastoma cells SH-SY5Y by analyzing the endo- and exo-metabolome, demonstrating that the metabolomics profile of cells pretreated with nicotine before the addition of Aβ peptide became more similar to that of untreated cells.

Keywords: *Pharmacometabolomics, Alzheimer's disease, nicotine, amyloid, NMR*

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mRNA-loaded Self-assembling Nanoparticles as Novel Vaccine Formulations

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Keywords: *self-assembling nanoparticles, mRNA, nucleic acid delivery, immunogenicity*

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Messenger RNA (mRNA) has shown a dramatic potential for the development of next-generation vaccines. To overcome some of the biopharmaceutical challenges that hamper mRNA administration (e.g., its rapid degradation in biological fluids and poor uptake into the target cells), nanocarriers such as lipid nanoparticles (LNPs) have been used in the two mRNA-based vaccines approved in 2021 [1]. In order to preserve their *in vivo* efficacy, mRNA-loaded LNPs need to be stored and transported at extremely low temperatures, which limits their widespread use. Alternative formulations for mRNA-based vaccines with long shelf life at 4 °C are therefore needed. Here, we aim to overcome these issues by leveraging the lipid self-assembling nanoparticle (SANP) technology for the development of novel mRNA-based vaccines. Lipid SANP formulations have shown remarkable biocompatibility, high RNA encapsulation efficiency and intracellular release [2]. SANP can be prepared by simple component mixing at room temperature immediately before use; this feature enables storage and transport of mRNA in lyophilized form, which ensures greater stability against degradation compared to freezing. As such, lipid SANP may be suitable for the formulation of vaccine platforms.

To investigate the feasibility of this strategy, we developed SANP encapsulating mRNA upon component mixing at room temperature by adapting a well-defined protocol established in our group [2]. The mRNA-CaP complexes were mixed with the cationic liposomes to obtain mRNA-SANP, which were characterized in terms of mean diameter, polydispersity index and zeta potential, mRNA encapsulation efficiency, colloidal stability up to 14 days post-assembly, stability against aggregation upon incubation in serum at 37 °C, and hemolytic activity. The ability of SANP formulations to induce an immune response *in vivo* was demonstrated following the administration of mRNA-SANP *via* intravenous (i.v.) or intramuscular (i.m.) injection at different mRNA doses and administration regimes; in this case, a mRNA encoding for the spike protein of COVID-19 was used. Importantly, no renal and hepatic toxicity was observed following mRNA-SANP administration. The expression kinetics of mRNA upon i.v. or i.m. injection was assessed at various time points by measuring the expression of an alkaline phosphatase encoded by the mRNA. We could detect alkaline phosphatase in the blood for up to 7 days post-administration regardless of the administration route. In conclusion, we have successfully developed mRNA-loaded SANP formulations with favorable colloidal properties and the ability to elicit the expression of mRNA-encoded proteins as well as an immune response *in vivo* with no organ toxicity.

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Alternative tools for rapid and high throughput assessment of transdermal passage

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Transdermal administration route represents an attractive alternative to conventional drug delivery systems such as oral or parenteral delivery [1]. On the other hand, the skin may be a route of exposure to contaminants, due to their widespread use in personal care products [2]. In both cases, it is essential to evaluate the skin degree permeation. So far, the methods available for studying the permeation of substances through the skin are mostly based on animal model membranes and are, in general, poorly reproducible and low to medium throughput, surrounding ethical issues [3]. To date, the assessment of skin permeation has been conducted prevalently by Franz-cell diffusion cells consisting of two compartments separated by animal skin, enabling the assessment of a substance's ability to penetrate the skin barrier [4]. Higher throughput, greener, animal testing-free, and alternative tools are been investigating by us. Biomimetic chromatography, exploited on stationary phases able to mimic biological components such as (phospho)lipids and proteins, can offer effectiveness in profiling solutes for their dermal permeation potential [5]. Recently, the predictive potential of the biomimetic chromatography has been coupled and exploited by the using of comprehensive two-dimensional liquid chromatography (2D LC), and applied with success to predict human intestinal absorption [6].The structure of the skin has been emulated by developing an analytical platform, based on comprehensive 2D LC, using a narrow bore octadecyl silyl stationary phase in 1D mimicking the subcutis, which is rich in adipocytes [7], and an immobilised artificial membranes phase in 2D, emulating the derma. Furthermore, the PermeaPad® system, a biomimetic, synthetic membrane for simulating passive mass transfer has been tested as well. The aim was to explore new methods able to reduce and/or replace animal tests[8, 9]. To validate both techniques, 50 pharmaceutical and cosmetic ingredients, whose passage through the skin was achieved by using the conventional Franz cell diffusion apparatus [10, 11] have been analysed.

Keywords: xenobiotics, biochromatography, skin permeation, bidimensional chromatography.

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Designing carbon nanoparticles-diatomite hybrids for wastewater remediation

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Many water sources become inaccessible due to their pollution, which limits their use in any sector, from human consumption to industry [1]. There are numerous anthropogenic sources of water pollution, the most abundant of which are releases from mining, industrial processes, pesticides and agricultural fertilisers [2]. In this framework, to reduce the amount of pollutants in wastewater the use of sorbent materials is gaining a lot of interest. A distinction can be made between natural and anthropic materials among the various materials commonly studied for their capture properties. Generally, natural materials are preferred, given their limited environmental impact and lower production costs, but functionalised natural materials, designed to improve their capture performances, are also receiving great interest [3].
In this work, the possibility of producing hybrid materials with improved adsorption properties was explored by combining a natural diatomite with hydrophilic carbonaceous nanoparticles (HNP) obtained by a nanostructured carbon black [4]. The HNP colloidal stability over a wide pH range, due to the presence of oxygen-functional groups (mostly carboxylic acids), is an obstacle to the filterability of exhaust particles at pH values superior to 3. The HNPs exhibit enhanced sorption behaviour toward heavy metals including rare earth metals [4,5] and the possibility to combine HNP with inorganic natural scaffolds opens to the development of adsorbents with improved capacities. A first attempt in this direction was realized by using silica nanoparticles [3]. The hybridization is a topic of great interest due to the possibility of synergistic enhancement and expansion of the adsorption performance increasing filterability and ease of recovery after use. To this aim, two hybrid diatomite-based particles loaded with 2 wt.% and 5 wt.% of HNP, were produced. All the materials were characterized by X-ray fluorescence, X-ray diffraction, scanning electron microscopy and Fourier transform infrared spectroscopy. In addition, the charge surface properties of the materials were also analysed by Z potential measurements. The proposed materials were tested for methylene blue (MB) adsorption at different pH solutions (with a particular focus in the range 7.0-9.0). The effects of MB concentration, contact time, and adsorbent mass of the materials were also investigated. The adsorption data were modelled by using the Langmuir equation. The kinetics parameters estimated for the MB adsorption with new hybrid materials were then compared with those of the raw material. As expected, the kinetic parameters are dependent on the degree of hybridization. Specifically, by increasing the carbon nanoparticles content, the rate of adsorption and the kinetic values increase and result strongly higher than those currently shown in the literature for this type of material. Overall an increase in the cyclability and the consequent process economics arises.

Keywords: *Diatomite, hydrophilic carbonaceous nanoparticles, adsorption kinetic, methylene blue.*

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Non-covalent Interactions with Proteins of Potential Vanadium Drugs: The Case of the Lysozyme/$V^{IV}O$-8-hydroxyquinoline adduct

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Vanadium complexes (VCs) have been suggested for the treatment of different diseases, such as diabetes and cancer. However, none of those compounds is currently used in clinics.[1] The use of VCs as drugs is primarily hampered by the limited knowledge of their transformation in the organism, which is related to the interaction with bioligands. In fact, the interaction of the active species of VCs with biological macromolecules, like proteins, is of great importance for their pharmacological activity.[2,3] Among the most promising VCs, $[V^{IV}O(8\text{-HQ})_2]$, with 8-HQ 8-hydroxyquinolinato as ligand, shows important biological activities. In this work the binding of the potential drug $[V^{IV}O(8\text{-HQ})_2]$ with the model protein hen egg white lysozyme (HEWL) was evaluated through: electron paramagnetic resonance (EPR), UV-visible, electrospray ionization-mass spectrometry (ESI-MS), X-ray diffraction (XRD), DFT and docking studies. The results of ESI-MS indicate that, under the experimental conditions (at pH 4.5), adducts are formed with both 1:1 V:ligand and 1:2 V:ligand species formed by 8-HQ, $[V^{IV}O(8\text{-HQ})]$/$[V^{IV}O(8\text{-HQ})(H_2O)]$ and $[V^{IV}O(8\text{-HQ})_2]$, with V in the +4 oxidation state. EPR spectra show that, under the used experimental conditions, two V-containing species coexist in solution and that they interact non-covalently and covalently with HEWL. These results agreed with XRD analyses: $[V^{IV}O(8\text{-HQ})(H_2O)]^+$ interacts covalently with Asp119 and is stabilized by H-bonds with Asn103 of a symmetry-related molecule and π–π contacts with Trp62 of a symmetry-related molecule; *cis*-$[V^{IV}O(8\text{-HQ})_2(H_2O)]$ interacts non-covalently with Arg128 and Lys96 from a symmetry mate. Additionally, the covalent binding of $V^{V}O_2^+$ to Asp48 and non-covalent binding of other V-containing fragments to Arg5, Cys6, and Glu7 is revealed. Finally, docking results suggest that the location of $[V^{IV}O(8\text{-HQ})(H_2O)]^+$ interacting with HEWL, in the absence of the interactions occurring at the protein-protein interface close to Asp119, should be close to Glu35 or Asp52 side chains. Globally, our results indicate that protein-protein stabilization could be a mechanism to drive unpredictable binding of metal compounds to proteins.

Keywords: *metallodrugs, protein metalation, biologically active V compounds*

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Process intensification for removal of new pharmaceutical compounds from water

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Nowadays, the diffusion of new emerging contaminants (ECs) (e.g. surfactants, pesticides, pharmaceutical compound, personal care product, etc.), present in the range concentration of ppt (ng/L) or ppb (µg/L), represent an environmental critical issue. Therefore, it is necessary find new technologies for drugs removal that escape by conventional wastewater treatment processes. Adsorption is the best technique to remove pollutants from water, due to its easy scalability, low cost, versatility, no undesired by-products generation and possibility to reuse the adsorbent into a new cycle of water treatment. Iopamidol (IPM) is a non-ionic X-ray contrast media, injected into the human body at high concentration (1.5 mol/L)[1], and relies into the aquatic environment in no-metabolized form [2]. The new challenge is to design a wastewater treatment plant capable to remove ECs efficiently, allowing the reuse of the aquatic matrix obtained. The best adsorbent for Iopamidol removal is activated carbon, so an adsorption study from batch to continuous flow was performed in this work. Ibuprofen (IBP) is a non-steroidal anti-inflammatory drug used in the treatment muscle pain and inflammation, which concentration in the wastewater is around 18–6297 ng/L[3]. Cellulose is a main component of natural plants, is the most abundant and renewable biomass resource on earth and is if pyrolyzed is a good adsorbent to remove IBP from water. For the intensification of the IPM and IBP adsorption process different kinetics were obtained using different devices, batch comparing a common stirrer with rotating bed reactor (RBR, Spinchem®), ultrasound probe merged into the aqueous solution and, finally, application of the continuous flow with and without ultrasound.
In this work several kinds of devices and technologies were tested aimed to find the best one for both iopamidol and ibuprofen adsorption. The use of an alternative stirrer, namely RBR, has shown a best IPM removal compared with a common stirrer, and a preliminary study was conducted to find the best working condition (e.g. flow rate, IPM feed solution concentration) for the system in continuous flow realized. At the same time the use of an ultrasound probe merged into ibuprofen solution gives an improvement of the cellulosic adsorbent performance due to the fragmentation in time so an increase of the liquid-solid mass transfer. IBP adsorption tests will be performed also in continuous flow using a similar IPM adsorption apparatus.

Keywords: *Emerging contaminants, wastewater treatment, process intensification, pharmaceutical compounds*
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New Promising Curcumin Mimics as Neurodegenerative Hallmarks Rescuers[1]

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Keywords: Natural products; Neurodegeneration; Aβ-amyloid; Proteasome

Proteins' misfolding and the formation of their aggregates is a common event to several human pathologies (Protein Misfolding Diseases – PMDs) and neurodegenerative disorders, as Alzheimer's Disease (AD). Recently, it has been observed that a decreased activity of Ubiquitin Proteasome System (UPS),[2] fundamental pathway of misfolded or damaged proteins, leads to an accumulation of the proteins that plays a key role in Protein Conformational Diseases (PCDs). Starting from the selected lead-metabolite Curcumin (**Cur**),[3] reported to have an unprecedented therapeutic potential in the pathophysiology of AD, but poor pharmacokinetics (PK), different approaches of drug discovery have been pursuing for the development of novel molecules capable both to interfere with protein misfolding processes and to enhance the activity of UPS. To create derivatives with better drug-like properties and inspired by the presence of common structural elements among small-ligand proteasome activators, we have designed, synthesized, and characterized a mini-library of novel Curcumin mimics by varying the two aromatic moieties and modulating the length and rigidity of the newly settled diamide spacers. These compounds will be functionally probed for their antiaggregating ability and to stimulate h20S proteasome, both crucial capabilities in restoring cellular proteostasis. Resulting structure-activity relationships will be used to implement the pharmacophore model to drive future structure optimization.

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Optimization of Ursolic Acid Enriched-Oleolytes from Annurca Apple with Potential Depigmentation Activity

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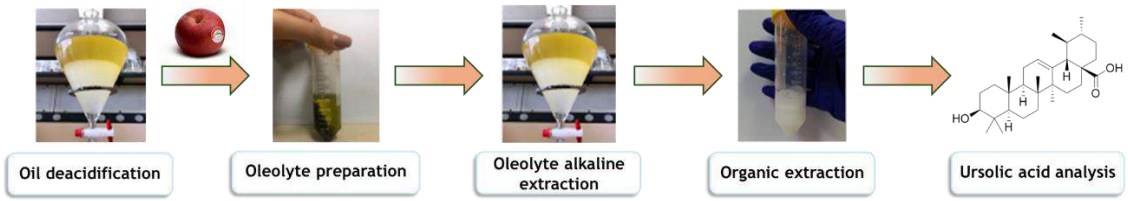
Ursolic acid (UA) is a pentacyclic triterpenoid carboxylic compound widely distributed in fruits, especially in apples. In recent years, this molecule has attracted considerable attention due to its functional activities, such as antioxidant, neuroprotective, anti-inflammatory, antibacterial, and depigmentation property [1]. The main aim of this project was the preparation of an enriched-UA oleolyte from Annurca Apple for the management of skin-hyperpigmentation disorders (e.g., melasma, age spots). The optimization of UA extraction was performed using sunflower oil as a lyophilic solvent and applying a Response Surface Methodology (RSM) statistical approach. The RSM analysis showed a maximum UA extraction yield of 784.40 ± 7.58 µg UA/mL of oleolyte under optimized conditions (68.85 °C as extraction temperature and 63 hours as extraction time). UA quantitative analysis was performed applying two consecutive alkaline and organic extractions. The HPLC-DAD-HESI-MS/MS analysis of the optimized oleolyte extract identified 23 and quantified 8 compounds, respectively [1]. To assess the potential of the enriched-UA oleolyte in the treatment of skin-hyperpigmentation disorders. Abnormal melanin production led to the skin hyperpigmentation disorder. Melanogenesis is mediated by the tyrosinase enzyme and is regulated by other factors, such as tyrosinase-related protein (TRP) ½ and microphthalmia-associated transcription factor (MITF) [2]. Therefore, we performed enzymatic and cells *in vitro* assays for the evaluation of the enriched-UA oleolyte activity on melanin production and the expression of MITF and (TRP) ½ factors in melanoma cell line. UA analytical standard and the enriched-UA oleolyte inhibit tyrosinase with an IC_{50} = 58.73 µM and 286.42 µg/ml, respectively. Tyrosinase inhibition was evaluated by a properly validated HPLC-FLD assisted enzymatic assay. Additionally, treatment of melanoma cells with the enriched-UA oleolyte (30 µg/ml) displayed a relevant reduction in melanin content (-20% *vs* CTR; $p < 0.0001$) and the expression of melanogenesis modulators TRP1 (-60% *vs* CTR; $p < 0.0001$), TRP2 (-15% *vs* CTR; $p < 0.05$), MITF (-30% *vs* CTR; $p < 0.05$).



Keywords: *Ursolic Acid quantification, Annurca apple, oleolyte, melanin, tyrosinase*

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Development of dual BRD9/HDAC hybrid ligands as novel epigenetic probes

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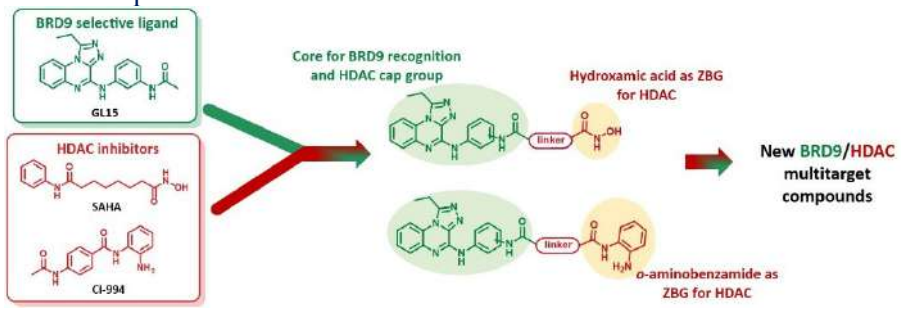
The chromatin reader bromodomain-containing protein 9 (BRD9), through the recognition of *N*-acetylated lysine (KAc) residues on histones, has a critical function in regulating the arrangement of chromatin and transcriptional programmes.[1] Specifically, this epigenetic protein has been receiving increasing attention since it controls the expression of oncogenes and anti-apoptotic proteins and its aberrant activity results in phenotypic alterations linked to various types of cancer.[2] The highly selective BRD9 inhibitors so far disclosed, feature a limited efficacy on the anticancer activity in cell environment, probably due to the presence of other proteins that can compensate for the blockage of BRD9.[3] Accordingly, the identification of a new class of multitarget compounds represent a yet unexplored investigation that would serve as a valuable *in vitro* tool. Herein we present an innovative strategy based on the design, efficient synthesis and preliminary biophysical evaluation of novel hybrid compounds targeting the epigenetic reader BRD9 and the eraser of lysine acetylation, the histone deacetylase (HDAC), also involved in key processes of cancer initiation and progression.[4] This valuable approach aims to disrupt synergistically the activity of these correlated proteins and to potentially enhance the anticancer effect in a cellular context. Importantly, the investigation of the intricate interactions between diverse epigenetic modifiers could enable the development of more potent and efficient anticancer compounds.



Keywords: *BRD9, HDAC, multitarget inhibitors, epigenetic therapy, organic synthesis*

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Molecular fingerprint by omics-based approaches in saliva from patients affected by SARS-CoV-2 infection

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The omics sciences based on advanced technologies and multidisciplinary expertise enormously contribute to figuring out the biological mechanisms underlying disease, eliciting particular interest, especially in clinical investigations. During the pandemic, scientists focused on developing analytical methodologies to monitor specific COVID-19 proteins in oro/nasopharyngeal swabs to find a method alternative to RT-PCR assay [1-3]. Since 2020, roughly 1,000 and 500 papers were picked out by matching COVID-19 and proteomics or metabolomics, respectively, as keywords in the Scopus research platform. Numerous studies were focused on the changes in the abundance of biofluid proteins or metabolites to investigate the organism's response to the viral infection. The saliva was often chosen as matrix proved to be a strategic biofluid owing not only to its appeal requiring a non-invasive sampling method but also to the capacity of the virus to invade epithelial cells of the oral mucosa and salivary gland ducts via ACE2 receptors [4]. Moreover, the correlation between the expression of COVID-19 infection (including fatal cases and patients with mild or low symptoms) and the saliva viral load was extensively debated [5, 6] suggesting to investigate the changes of metabolic pathways following the viral infection independently to the viral load in saliva or oro/nasopharyngeal swabs.
In the present paper, the case-cohort samples consisted of 63 saliva from hospitalized COVID-19 patients and 30 from healthy controls collected to assess the biochemical alterations following the infection by integrating proteomics, peptidomics, and metabolomics approaches. An untargeted LC-MS/MS was used for proteomics and peptidomics analysis, while an LC-MRM/MS method was developed to monitor the panel of 20 amino acids without performing any derivatization step. The levels of 77 proteins were significantly different in COVID-19 patients: among these, 7 proteins were found only in saliva from patients with COVID-19, 4 were up-regulated and 3 were down-regulated at least 5-folds in saliva from COVID-19 patients in comparison to controls. The analysis of proteins revealed a complex balance between pro-inflammatory and anti-inflammatory proteins and a reduced amount of several proteins with immune activity that possibly favors the spreading of the virus. Such reduction could be related to the enhanced activity of peptidases induced by the infection that in turn caused an altered balance of free peptides. Indeed, on a total of 28 peptides, 22 (80%) were differently expressed in SARS-CoV-2 and control subjects. The multivariate analysis of such peptides permits obtaining a diagnostic algorithm that discriminates the two populations with high diagnostic efficiency. Finally, the metabolomic fingerprint of salivary amino acids suggests an alteration of metabolic pathways as a result of viral infection, mainly involving threonine and alanine.

In conclusion, the present study defined a set of molecules to be detected by an integrated approach based on tandem mass spectrometry useful in revealing biochemical alterations involved in the pathogenesis of such a complex disease to obtain a set of putative biomarkers useful for non-invasive diagnosis.

Keywords: *proteomics; peptidomics; metabolomics; amino acids; LC-MS/MS*

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Biomimicking amyloid aggregates for self-assembling biomaterials

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Studies carried out in the last decades have demonstrated that, due to non- covalent interactions, proteins and peptides can organize into highly stable supramolecular assemblies, including amyloids and nanotubes. In particular, by exploiting the fast-growing amyloid-related structural information present in the Protein Data Bank, heterotypical regions can represent a database of bio-inspired self-assembling sequence. To verify the feasibility of this approach, a sequence of transthyretin (namely P3, residues 14-32) was selected as model. P3 consists of two strands (P3A and P3B) linked by a short loop of four amino acids (RGSP) P3 arranges in a β-harpin, stabilized by P3A and P3B interactions.
With the aim of mimicking the P3 supramolecular arrangements, P3, P3A and P3B sequences were synthesized and multiscale characterized their supramolecular behavior.
Stimulatingly, the systems formed by the co-aggregation of P3A and P3B is able to reproduce the native P3 sequence structuration. All the peptides were also found able to generate self-supporting hydrogels after a solvent switch procedure, thus demonstrating that heterotypical regions can inspire novel biomaterials.

Keywords: peptide self-assembling, biomaterials, amyloid-like aggregates, heterotypical sequences.

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Development and validation of an eco-compatible UV-Vis spectrophotometric method for the determination of Cu^{2+} in aqueous matrices

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The field of contaminant analysis is generally very strictly regulated and not very open to innovation in the analytical procedure. Nevertheless, since the green chemistry (GC) principles were proposed, efforts have been spent to the development of more sustainable protocols, giving rise to the Green Analytical Chemistry (GAC). Concerning the Cu^{2+} determination, the Italian Water Research Institute (IRSA) has proposed an ICP-OES and a spectrophotometric analysis (based on the formation of a Cu complex with oxalyldihydrazide) as reference methods [1]. However, the application of oxalyldihydrazide-based method shows some limitations, due to the significant number of steps and harmful reagents required, making this method disrespectful of GAC principles [2]. In this context, a new spectrophotometric method was developed with the aim to provide a rapid and simple strategy for the copper determination with short time and low-cost analysis. For this purpose, the iminodisuccinic acid (IDS), a biomass-derived and biodegradable ligand, has been employed for the formation of a Cu-IDS complex, characterized by an intense absorbance peak (710 nm). The formation of such colourful complex has been exploited for the first time in the development of a spectrophotometric method for the determination of Cu^{2+} in aqueous matrices. In detail, the developed method shows a remarkably wide linear range (6.3–381 mg L^{-1}) and low limit of detection (LOD) (1.43 mg L^{-1}). Moreover, the analyte determination trough this analytical protocol is not affected by either metal cations (*e.g.* Ca^{2+}, Mg^{2+}, Fe^{3+}) or the complexity of the investigated matrices, including drinking water, river water and wastewater [3]. Furthermore, the AGREE assessment tool [4] was used for a quantitative comparison between the proposed method and reference one, on the basis of their agreement with the green analytical chemistry principles. The results showed the lower environmental impact of the proposed method and the suitability of this novel approach for Cu^{2+} in water matrices.

Keywords: *Copper, Spectrophotometry, Green Analytical Chemistry, AGREE*

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Harnessing the isoxazole core to achieve hybrid LIFR-FXR modulation

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The leukaemia inhibitory factor (LIF) is a cytokine belonging to the IL-6 family. LIF signalling is started by binding to the heterodimeric complex formed by the LIFRβ and Gp130 proteins and leads to JAK1/STAT3 activation. LIF plays several roles in the modulation of many pathophysiological processes. Many studies have proven its involvement in tumorigenesis, thus prompting LIFR inhibition as a potential therapeutical strategy for cancer.[1] Moreover, other studies suggested the possible yet uncertain involvement of LIF-LIFR axis in metabolic regulation and consequently in metabolic disorders like NAFLD and fibrosis.[2] In this context, the Farnesoid X receptor (FXR) is a ligand-activated factor involved in bile acids biosynthesis and glucose and lipid homeostasis. Many FXR agonists have been shown to exert protective effects on *in vivo* NAFLD/ NASH models and some of them have reached advanced clinical studies.[3] Moreover, FXR activation suppresses hepatocellular carcinoma, and its loss also promotes the progression of colorectal cancer.[4] Therefore, the possibility of exploiting the hybrid modulation of both LIFR and FXR could be a potential synergistic approach for the treatment of either metabolic disorders or cancer. To this end, we investigated whether the 3,4,5-trisubstituted isoxazole moiety, a renowned chemical class of FXR agonists, could somewhat possess sufficient structural motifs to target LIFR. We present first-in-class hybrid LIFR inhibitors and FXR agonists with the best candidates being endowed with good pharmacological activity on HepG2, Raw264.7 and HsteC cell lines.

Keywords: *cancer, isoxazole, NASH, FXR, LIFR*

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2D-NMR in structural elucidation of specialized metabolites from Trichoderma spp. bioactive fractions

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Endophytic fungi establish physiological relationships with the hosts plants without causing evident damage. This symbiotic relationship can be beneficial for both participants (Selim et al., 2012). Trichoderma species are filamentous fungi described for the first time in 1794 (Zhang et al 2021), they produce a large spectrum of specialized metabolites with remarkable biological activities, including biocontrol of plant pathogens or nematocidal activity (Moo-Koh et al 2022). One major hurdle in agricultural crop production is the capability of parasitic nematodes, such as Meloidogyne spp., to cause considerable damage and losses of a range of crops. These nematodes invade plant root tissue and determine the formation of galls or "knots" which disrupt the plant's ability to absorb water and nutrients, thus resulting in stunted growth, yellowing, and wilting of the host (J.N. Sasser 1980).
Based on this background, the aim of this study was to investigate the production of bioactive metabolites by one Trichoderma parceramosum and one Trichoderma citrinoviride strains and to individuate metabolites that can be potentially used as nematocidal agents. The hydroalcoholic extracts of fungal cultures in Solid State Fermentation (SSF) were subjected to extraction, first with n-hexane and then with dichloromethane. The more polar fractions, trough chromatographic techniques, were separated into fractions which were tested for nematocidal activity against Meloidogyne incognita. From the purification of the most active fractions, one phenolic compound and heterocyclic lactones were isolated. 1D-NMR experiments and exhaustive 2D-NMR investigations allowed to determine the structure of the pure compounds.

Keywords: *Trichoderma spp; nematocidal activity; chromatographic purification, NMR, toxic fungal metabolites.*

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Bile acid derivatives as LIFR/LIF inhibitors

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Pancreatic cancer (PC) is the seventh leading cause of cancer-related deaths in industrialized countries. Leukemia Inhibitory Factor (LIF) is a pleiotropic member of interleukine (IL)-6 cytokine family, overexpressed in a variety of solid tumors (including pancreas), promoting cancer cell proliferation.[1] There are no LIFR antagonists approved for clinical use. In this context, my research group previously identified Mifepristone, that is a medication typically used to bring about a medical abortion during pregnancy, as potential LIFR antagonist. Considering the recent pharmacological results of natural and semi-synthetic bile acid derivatives on LIF/LIFR axis[2] our aim was to modify the steroidal core of Bile Acids (BAs), to generate a new library of LIF antagonists. Another well-known LIF antagonist reported in the literature is compound EC359, which presents many structural similarities to Mifepristone, supporting our hypothesis of steroidal-scaffold based molecules to reduce LIF activity.[3] Basing on this background and combining the structure-activity relationship of bile acids with the steroidal structure of known LIF antagonists, we developed a synthetic library of BAs derivatives modified in position 11 with different aromatic substituents in order to obtain a first set of C11 modified derivatives, to evaluate the influence of these type of modifications on LIF/LIFR activity. Synthesized compounds will be subjected to a preliminary pharmacological analysis on LIF/LIFR pathway using an Alphascreen cell-free assay. A deep in vitro and in vivo pharmacological evaluation on the best molecules, led us to identify new chemical entities for the treatment of several type of solid tumors.

Keywords: bile acid, leukemia inhibitory factor receptor, pancreatic cancer.

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Compatibilization of Isotactic Polypropylene (iPP) and Polyethylene (PE) with PP-based Block Copolymers

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In this work, the effect of addition of commercial polyethylene (PE)-*b*-polypropylene (iPP) di-block copolymer (PE-*b*-iPP) to PE/iPP blends is reported. It is well known that PE/iPP blends are generally thermodynamically immiscible and hardly separable at the end of their life cycle, once rescued from landfills.[1] These blends show poor mechanical properties compared with those of the neat components. The addition of less than 5% PE-*b*-iPP copolymers as compatibilizers is reported to improve their mechanical properties and the adhesive strength at iPP/PE interface.[2] The aim of the study is to optimize the mechanical properties of iPP/PE blends to create opportunities for more effective recycling of mixed iPP and PE waste materials into equal or even higher value products (upcycling), without resorting to their separation.
The study is carried out on PE/iPP blends at 70/30 wt% composition, in order to mimic the approximate PE/iPP composition ratio of the urban plastic waste. The PE-*b*-iPP block copolymers used as compatibilizer are commercially available at Dow® Chemical Company with the tradename INTUNE™.[3] The INTUNE™ copolymers are obtained through Coordinative Chain-Transfer Polymerization (CCTP), an economic alternative to the living polymerization, which benefits from the regenerative chain transfer reaction in the presence of an appropriate chain transfer agent (CTA), like $ZnEt_2$ or $AlEt_3$, in the reaction media.[4]
It is shown that the addition of INTUNE™ copolymers provides good mechanical properties to iPP/PE blends already at 3-5 wt% content. A possible compatibilization mechanism is proposed.

Keywords: *isotactic polypropylene (iPP), polyethylene (PE), polyolefin blends, compatibilization, olefin block copolymers*

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Analysis of post-translational modifications (phosphorylation and N-Glycosylation) in proteins extracted from Tempera paintings

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The use of protein matrices (e.g., milk and eggs) [1] as binders for tempera painting is one of the main causes of the marked vulnerability to degradation phenomena during the aging of artifacts made with this technique. Their identification and characterization remain a challenging task for several reasons: their complex and variable composition, their simultaneous presence, and the presence of degradation products due to aging [2]. The proteins most commonly found in such matrices are characterized by the massive presence of post-translational modifications (phosphorylation and N-glycosylation). These chemical modifications profoundly affect the physicochemical properties of the proteins to which they are bound, and thus a relationship between these types of modifications and possible degradation processes can be hypothesized.
Artifacts are exposed to the weather and subjected to the influence of environmental parameters [3]. Physical, chemical, and biological phenomena alter the constitutive materials, inducing changes both in their compositional and structural characteristics. In this context, the purpose of the project is to develop and optimize an analytical method for the extraction and characterization of post-translationally modified proteins contained in tempera binders.
The procedure was optimized on specimens expressly prepared according to old recipes [4] by mixing different types of binders with a pigment (yellow ochre, iron (III) hydroxide) [5] and submitted to artificial aging (simulating photo-oxidative stress conditions). A comparison of data obtained by fresh and aged samples was performed to eventually identify differences in the profile of identified PTMs attributable to adopted aging conditions; finally, the same procedure was applied to a 16th-century painting, "*Battesimo di Cristo*," and to the pictorial layer of a 13th-century wooden sculpture, "the Great Polychrome Crucifix.". The procedure was based on protein extraction and hydrolysis, advanced biomolecular mass spectrometry methodologies, and selective methods for the identification of PTMs. The data thus obtained will expand the molecular characterization of Tempera-painted surfaces, thus leading to a deeper knowledge of the artifacts and facilitating the development of new diagnostic and restoration methodologies. Moreover, the results reported in this study show how, under certain aging conditions, these modifications are subject to considerable variability.

Keywords: *tempera painting, mass spectrometry, post-translational modifications*

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Il Contributo dei Giovani Chimici in Campania
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Transition Metal-Catalysis for the Manipulation of Molecular Structure

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Transition metal-catalysis has revolutionized organic synthesis, providing novel reactivity modes to activate previously unreactive functional groups or unlocking new transformations and selectivities.

Among the most recent advances in the field, remote functionalization and cross-coupling stand above. In this context, I begun with the Pd-catalyzed remote functionalization of alkenyl alcohols to obtain highly versatile α,ω-dicarbonyl compounds[1] and then combined olefin isomerization with subsequent functionalization steps; these multimetallic, one-pot protocols gave access to densely functionalized molecules, avoiding the cost- and resource-inefficient isolation of unstable intermediates. Ni-catalyzed cross-coupling of challenging vinyl methyl ethers[2] and Cu-catalyzed protoboration of vinyl (hetero)aryls[3] are only two examples of these transformations.

Subsequently, the multimetallic approach has been substituted with more appealing single-catalyst or metallaphotoredox strategies and the manipulation of other ubiquitous, yet challenging, strong C(sp^3)–X bonds has been targeted:

- C(sp^3)–N bond: the Ni-catalyzed hydroalkylation of olefins has been developed using aliphatic amines derivatives as alkyl-precursors.[4]
- C(sp^3)–H bond: a metallaphotoredox approach has been used for the α-alkylation and α-arylation of aliphatic amides.[5]
- C(sp^3)–O bond: the Ni-catalyzed cross-coupling of cyclic acetals has been developed, allowing the manipulation of these moieties, usually regarded only as protecting groups.[6] Aiming at reconsidering protecting groups as synthetic handles for selective transformations, the deoxygenative oxidation – concomitant cleavage of one C–O and formation of another C=O bond – of mono-protected diols (prepared *in situ* or *ex situ*) have been targeted *en route* to aliphatic ketones, exploiting a *Spin-Center Shift* (SCS) mechanism.[7]

Keywords: *cross-coupling, remote functionalization, transition metal-catalysis, photoredox, metallaphotoredox*

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Targeting METTL3-14 degradation by PROTAC technology: design, synthesis and biological evaluation of new promising library of degraders[1]

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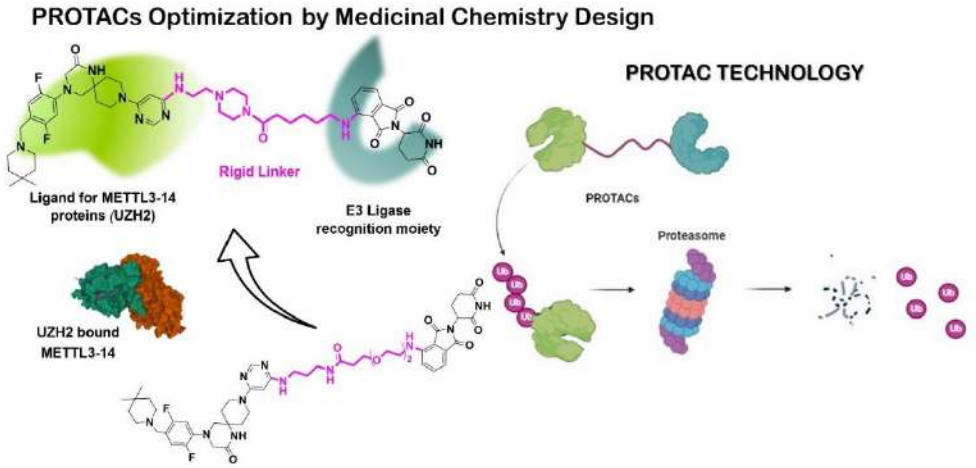
Recent studies of the ‘‘epitranscriptome’’ reveal important roles of different RNA modifications in cancer.2 Methylation of adenine N6 (m6A) is the most frequent RNA modification, and it has been first associated with tumorigenesis in Acute Myeloid Leukemia (AML) and with other types of blood cancers and solid tumors. On mRNA, it is catalyzed by the core writer complex formed by two methyltransferase-like proteins, METTL3 and METTL14. Here, we disclose the first PROteolysis TArgeting Chimeras (PROTACs) for proteins involved in epitranscriptomic regulation harnessing the ubiquitin-proteasome system (UPS) to degrade a target protein. Based on the crystal structure of the METTL3−14 complex with a potent and selective small-molecule inhibitor (UZH2), we report the design, synthesis, and biological evaluation of new PROTACs (Figure 1).3 To modulate the physicochemical properties of resulting PROTACs, a variety of linkers and functional groups have been investigated featuring PEG- or alkyl-based linkers of different lengths and rigidity. The formation of the ternary complex was validated by a FRET-based biochemical assay and an in vitro ubiquitination assay. The PROTACs that promote a more significant degradation of METTL3-METTL14 are characterized by a rigid “handle” (benzyl, piperidine, and piperazine) and a longer linker.



Keywords: *PROTACs, UPS, Epitranscriptome, METTL3-14*
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A Multidisciplinary Strategy for the Identification of a Novel Thiadiazolopyrimidone Targeting Annexin A6

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In recent years, a growing interest in thiadiazolopyrimidine derivatives made them promising scaffolds for the development of new therapeutic agents.[1] This study delves into the synthesis and interactome characterization of a novel bioactive thiadiazolopyrimidone compound (referred to as compound **1**), which exhibits cytotoxic effects on HeLa cancer cells. Specifically, starting from a small pool of synthesized thiadiazolopyrimidones, a comprehensive approach has been employed to investigate the most biologically active compound. Functional proteomics techniques and bio-orthogonal methods allowed us to identify a potential biological target Annexin A6 (ANXA6). Moreover, the impact of compound **1** on migration and invasion processes regulated by ANXA6 modulation was established (**Figure 1**).[2] The discovery of compound **1** as the first ANXA6 protein modulator marks a noteworthy breakthrough, providing valuable insights into the role of ANXA6 in cancer biology. This finding also sets the stage for the development of innovative anticancer treatments.

Keywords: *thiadiazolopyrimidones, annexin A6, functional proteomics, SPR, synthesis.*

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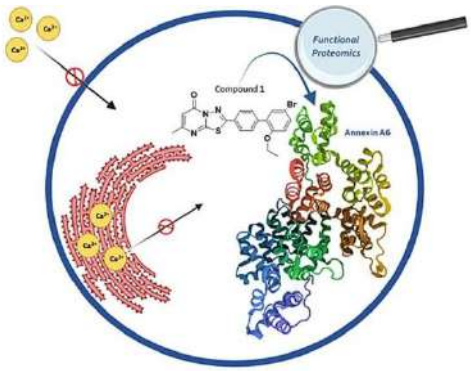

Figure 1: Identification of compoud 1 as the first modulator of ANXA6.

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GC analysis and microbiological analysis of lemon essential oils stored for 25 years

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Essential oils, also called “essences”, are natural compounds of plant origin. They are characterized by the presence of volatile substances at room temperature which give the product different odors and fragrances [1]. Characteristics and properties of each essential oil may vary based on the type of mixture that constitutes it, although it is possible to identify some characteristics common to all essential oils. From a chemical point of view, these substances are characterized by a marked volatility (presence of low-boiling compounds) and a very complex composition based mostly on low molecular weight compounds [2,3], among which the most studied and important are terpenes, such as limonene, present in fruits of the citrus family. The uses of essential oils are multiple, ranging from cosmetic use to food formulations, but also for therapeutic purposes, as they possess, albeit to a variable extent, a certain antimicrobial activity [4]. Clearly, in order to preserve their characteristics and properties, it is very important that essential oils are stored correctly. Therefore, in this work the shelf life of some samples of essential oils extracted from lemons from Southern Italy was evaluated. For this purpose, commercial samples of essential oils adequately preserved for 25 years were examined, a period which greatly exceeds the shelf life normally suggested to keep the main properties of the oils themselves unchanged. Accordingly, the samples were subjected to a series of determinations, such as GC-FID, NMR and microbiological analyses, and compared with freshly produced oils.

Keywords: *Essential oil, natural products, gas-chromatography, NMR, antimicrobial activity.*

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Metabolomic Analysis of *Malus domestica* (Suckow) Borkh. Varieties From Molise Region (Italy) by NMR Spectroscopy

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Apples are one of the most extensively produced and consumed fruits worldwide [1]. They represent an important source of bioactive compounds like polyphenols which are responsible for their antioxidant and anti-inflammatory properties and possess a role in the prevention of degenerative diseases [2]. This work aims to the valorization of several autochthonous varieties belonging to *Malus domestica* (Suckow) Borkh. of Molise region (Italy): even if the nutritive features of these species are well known, the knowledge of the Molise region products, like apples and pears, is still incomplete than the other Italian regions (e.g., Annurca apple of Campania region). Indeed, the metabolic profile, with primary and secondary metabolites, can vary in the same species qualitatively and quantitatively if it grows in different environmental conditions, including the type of soil [3]. The metabolomic profile will be assessed through a quali-quantitative determination of the constituents of each selected variety by 1D and 2D NMR Spectroscopy and chemometric techniques (PCA and PLS-DA) will be applied in order to observe differences among the varieties. Furthermore, the total phenolic, flavonoids, and condensed tannins content will be assessed, and in vitro antioxidant activity will be evaluated by using DPPH scavenging activity, the ABTS scavenging assay, and ferric-reducing antioxidant power (FRAP). The investigation of the antioxidant activity will be an index of the potential application in cosmetics of the selected varieties of apples from Molise region with the goal to obtain a cosmeceutical formulation.

Keywords: *Malus domestica, NMR, metabolomics, chemometrics, metabolic profile*

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Cross-linking Mass Spectrometry to decipher Cell Plasma Membrane Interactome

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Functional interactions among proteins are fundamental to cell biology. Constituting ~30% of the mammalian proteome and 60% of all drug targets, plasma membrane proteins (PMPs) playing important roles in the modulation of diverse molecular processes, including transport, signal transduction, endocytosis and secretion [1]. Revealing the interactome of PMPs under different biological conditions is an unbiased approach to depict regulatory pathways controlling cell behaviour. Despite the biochemical impact, knowledge gaps regarding protein-protein interactions (PPIs) at PM persist.
Recently, cross-linking mass spectrometry (XL-MS) has emerged as a powerful tool for interactions discovery and characterisation, driving the enlightenment of novel binding partners otherwise undetected. Covalent linkages of two amino acid residues of proteins or within complexes, in close proximity, can be identified by MS, thus providing structural insights or unravelling interaction dynamics [2].
Accordingly, we applied the XL-MS strategy to map, on a system-wide scale, the PMPs networks of He-La cancer cells, as cellular reference model. PM- fractions were enriched from total cell lysates cross-linked in both PM intact and disrupted status and subsequently analysed via label-free nLC-MS/MS. Based on all detected inter-links, we constructed a global network composed of unique residue-to-residue pairs originating from more than 300 proteins assigned to the PM compartment. The comparison to known interactions revealed an overlap of 25%, confirming the accuracy of our dataset. The remaining cross-links were identified for proteins which are currently not described as interacting with each other, presenting potentially *de novo* partners.

Keywords: *Cross-linking Mass Spectrometry, XL-MS, membranomics, interactomics, protein-protein interactions, regulatory networks, protein dynamics*

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New Aβ(1-42) ligands from anti-amyloid antibodies: Design, synthesis, and structural interaction

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Alzheimer's disease (AD), is the most common neurodegenerative disorder of the aging population resulting in progressive cognitive and functional decline. Accumulation of amyloid plaques around neuronal cells is considered a critical pathogenetic event and, in most cases, a hallmark of the pathology. In the attempt to identify anti-AD drug candidates, hundreds of molecules targeting Aβ peptides have been screened. Peptide molecules have been widely explored, appreciating chemical stability, biocompatibility, and low production cost[1]. More recently, many anti-Aβ(1-42) monoclonal antibodies have been developed, given the excellent potential of immunotherapy for treating or preventing AD. Antibodies are versatile ligands that bind a large variety of molecules with high affinity and specificity; however, their extensive therapeutic application is complex and requires huge economic investments[2]. Novel approaches to identify alternative antibody formats are considered with great interest. In this context, taking advantage of the favorable peptide properties and the availability of Aβ-antibodies structural data, we followed an innovative research approach to identify short peptide sequences on the model of the binding sites of Aβ(1-42)/antibodies. WAibH and SYSTPGK were designed as mimics of solanezumab[3]/crenezumab[4] and aducanumab[5], respectively. Circular dichroism and nuclear magnetic resonance analysis reveal that the antibody-derived peptides interact with Aβ(1-42) in the soluble monomeric form. Moreover, AFM microscopy imaging shows that WAibH and SYSTPGK are capable of controlling the Aβ(1-42) aggregation. The strategy to identify WAibH and SYSTPGK is innovative and can be widely applied for new anti-Aβ antibody mimicking peptides.

Keywords: *Alzheimer's disease, peptides, Nuclear Magnetic Resonance*

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Targeting c-Myc: Discovery and Enhancement of New Diphenyl Urea-Based Inhibitors for Cancer Treatment

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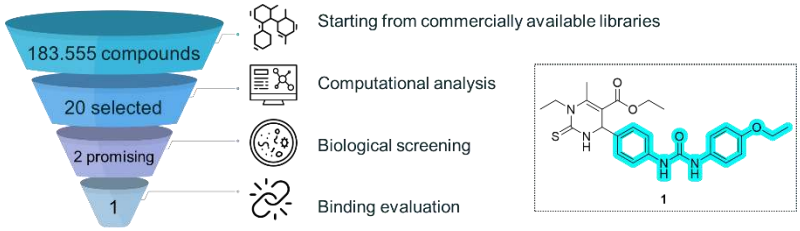
The proto-oncogene c-Myc is a pivotal player in cellular regulation, influencing proliferation, differentiation, and apoptosis processes. However, it is also constitutively and aberrantly expressed in over 70% of human cancers. Because of its central role in promoting cancer-related processes, c-Myc has become an attractive target in cancer therapy, but no drugs are clinically available yet. c-Myc has long been considered undruggable due to its nuclear localization, absence of a well-defined ligand binding site, and a physiological role essential for maintaining normal tissues. Besides, we embraced the challenge of investigating c-Myc and we discovered a new promising bioactive small molecule via a multidisciplinary approach: starting from 183.555 compounds, guided by computational studies, we conducted a biological and biophysical screening of twenty selected molecules, to successfully identify compound **1** as a novel inhibitor of c-Myc. Based on this preliminary discovery, we designed and synthesized a focused set of diphenyl urea derivatives to explore the structure-activity relationship around the diphenyl urea moiety, which seems to be essential for the interaction with c-Myc. Furthermore, our interest in Myc-protein has driven us to extend the strategies to target c-Myc in cancer cells by evaluating these novel bioactive molecules not only as direct inhibitors, but also as binders in targeted protein degradation technology applications.[2] To conclude, this comprehensive approach integrates chemical synthesis, computational study, and biological and biophysical assays, to advance our understanding and develop innovative potential therapeutical strategies.



Keywords: *drug discovery, computational studies, SPR analysis, biological evaluation, chemical synthesis.*

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Rational design of novel peptidomimetics against influenza a virus: biological and computational studies

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The effective treatment of Influenza virus infection is still an unmet goal. Even though some antivirals are available, the alarming increase in virus strains resistant to them highlights the need to find new drugs.[1] An ideal target for new anti-influenza therapy should be a viral component, whose function is essential for virus infection. In this contest, the influenza A virus hemagglutinin (HA) represents a very promising target.
Previously, we identified two tetrapeptides, SKHS (1) and SLDC (2), derived from bovine lactoferrin (bLf) C-lobe fragment 418-429, which were able to bind HA and inhibit cell infection in a concentration range of picomolar.[2,3]
Considering the above highlighted, the aim of this study was to synthesize a new library of peptidomimetics active towards influenza virus. In order to test their ability to bind HA, we carried out a preliminary screening by biophysical assays such as surface plasmon resonance (SPR) and the orthogonal immobilization-free microscale thermophoresis (MST) assays. Biological and computational studies on most interesting compounds were carried out.[4]
All applied methods agreed upon the identification of a N-methyl peptide, S(N-Me)LDC, able to bind hemagglutinin with high affinity and inhibit influenza virus hemagglutination and cell infection at picomolar concentration. This small sequence, with high and broad-spectrum activity, represented a valuable starting point for the design of new peptidomimetics. This work opens the way to new perspectives for the development of new anti-influenza drugs.

Keywords: *biophysical assay; docking; hemagglutinin; influenza; peptidomimetic.*

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Valorization and Metabolomic Analysis of Trub as a Sustainable Resource for the Pharmaceutical and Food Sector: A Circular Approach

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The widely recognized concept of circular economy[1] is based on the use of waste products derived from existing production processes as raw materials. This study focuses on companies whose processing waste is of plant origin, as they can be valued for their content of molecules, including secondary metabolites of potential interest in various sectors. The object of this study is the Trub[2], a by-product derived from the beer production process that contains waste from malt, hops, and yeasts. The analysis of Trub aims to evaluate both the presence of Xanthohumol and other polyphenols, of high economic interest[3], and to deepen its metabolomic profile. To contextualize the metabolomic landscape, initially an extraction phase was conducted on the matrix (Trub), using hydroalcoholic solutions at various concentrations to evaluate their extraction efficacy towards polyphenolic compounds and solutions at different polarities to evaluate all secondary metabolites included[4][5]. Afterwards an environmentally friendly extraction method was employed, such as subcritical water microwave extractors, to evaluate extraction efficacy comparatively and find alternatives to the use of common laboratory solvents with high environmental impact. The extracts subsequently have been analyzed using high-performance liquid chromatography combined with high-resolution mass spectrometry (LC-HMRS). Finally, bioinformatic techniques have been integrated such as Molecular Networking (MN)[6] , in order to evaluate the entire metabolomic profile. In conclusion, the importance of this research lies in the possibility of opening up research towards low environmental impact matrices, as they are already present in the production process, which may result in an integral resource of secondary metabolites with potential pharmaceutical interest and in the Food and Health sector, complemented by the implementation of more environment-friendly analysis methods. The presentation will showcase the results of different extraction methods and their comparison.

Keywords: Trub, circular economy, secondary metabolites, extraction methods, molecular networking, polyphenols.

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A rapid liquid chromatography/mass spectrometry method to identify promising protein biomarker of COVID-19 infection

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The investigation and explanation of the effects of Covid-19 pandemic waves on health are fundamental to design effective policy responses, apply new technologies and support healthcare investments directed to minimize the negative impact in society of recurring COVID-19 outbreaks and of other epidemics similar to the COVID-19[1]. The study of inflammatory status from literature data appears to carried out by ELISA (enzyme-linked immunosorbent assay), part of the enzyme immunoassay techniques. The ELISA method aims to detect and identify (both qualitatively and quantitatively) a specific substance within a sample, based on the use of antibodies labeled with an enzyme (usually peroxidase), indeed the resulting conjugates have both immunological and enzymatic activity. Through ELISA, however, only information regarding the presence or absence of certain molecules can be obtained, exploiting the affinity of an antibody for entire portions of a protein: this aspect is quite crucial as it allows characterization at the molecular level of the analyte of interest. Mass spectrometry is a methodology that is currently gaining ground especially in metabolomics analyses, but there are also enormous steps forward for proteomics. The untargeted approach allows to verify the expression of an entire genome while a targeted approach allows to evaluate the variations of certain pathways with greater selectivity, specificity and sensitivity[2]. What was done in the study presented is a LC-MRM/MS analysis on sera from women suffering from covid for the monitoring of target proteins of inflammatory and coagulative processes, altered in COVID19. The objective is to overcome the limitations of ELISA tests, such as the requirement for a distinct antibody for each protein and provide a unique qualitative and quantitative analysis capable of monitoring the whole set of proteins while cutting down on time and costs. A panel of 51 proteins involved in coagulation cascade and in inflammatory processes was selected to build up the MRM/MS method. Among these proteins, 32 allowed to discriminate Covid-19 and healthy control samples by demonstrating a p-value<0.05. The trend in protein content is comparable with published data regarding inflammatory processes due to Covid-19 infection and other pathologies.

Keywords: Covid 19, mass spectrometry, targeted analysis, infectious disease

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Silibinin-loaded amphiphilic nanoparticles: A promising drug delivery system for lung cancer therapy.

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Lung cancer ranks second globally in cancer-related mortality. In 1950, Doll and Hill identified tobacco smoking and environmental pollutants as significant risk factors[1]. Silibinin (SLB) is a polyphenolic flavonoid extracted from the seeds of Silybum marianum, demonstrating potent anti-carcinogenic activity against various carcinomas, including that to lung[2]. Despite its significant therapeutic potential, SLB is hindered by its poor water solubility and limited oral bioavailability[3]. To overcome these issues, the use of biodegradable NPs for encapsulating SLB was investigated to enhance its solubility and bioavailability. This design aims to utilize the enhanced permeability and retention (EPR) effect for passive tumor targeting[4]. NPs were made by a nanoprecipitation technique. Size, zeta potential (ZP), morphology, encapsulation efficiency and thermal properties were studied. For 30 days, the physical stability of all NPs was investigated. In vitro experiments were conducted to model the release mechanism of SLB from the NPs. In addition, the efficacy of SLB-encapsulated NPs was tested in three human cell lines: HI299, HI975 and H358. The NPs were spherical and around 110 nm in size, with ZP values ranging from -20 to -27 mV, indicating a hydrophilic PEO coating. DSC results showed SLB in a molecularly dispersed state within the NPs. For high SLB loading, the release was mainly diffusion-driven. Cell experiments revealed enhanced bioactivity against H1299, H1975, and H358 cells compared to free SLB when loaded in NPs. Based on these results, these NPs enhanced the bioactivity of SLB in lung cancer cells. However, quick release rates were observed, highlighting the need for sustained release in targeted therapies. Mathematical modelling indicated that modifying SLB concentration within the NPs could influence the release mechanisms. Future research will focus on optimizing formulations for extended SLB delivery.

Keywords: *Silibinin; amphiphilic nanoparticles; PLGA; poloxamers; lung cancer cells*

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Comparative life cycle assessment of different synthetic routes of ZIF-8

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The environmental issues we have been facing in the latest years are well-known to all: global warming, pollution and waste accumulation are the undeniable evidence of a wrong management of earth resources and lack of effective regulations. Meanwhile, the worldwide scientific community is increasingly aware of its decisional role and the current trend is to direct industry and institutions towards more sustainable choices: in this context, Life Cycle Assessment (LCA) methodology comes to our aid. LCA is regulated by the International Standardization Organization (ISO) through ISO 14040:2006 and 14044:2006 [1] regarding respectively Principles and Framework and Requirements and Guidelines of the procedure. This tool allows to associate environmental impacts to the whole manufacturing cycle, from the supplying of raw materials to the product end-of-life. In this work an example of *"cradle to gate"* LCA is given. The *functional unit* is 1g of ZIF-8, a versatile and widely employed material belonging to the ZIF (Zeolitic Imidazolate Framework) class. ZIFs are hybrid materials between MOFs and zeolites, and they have captured the attention of the scientific world for their exceptional features, such as crystallinity, porosity, as well as high chemical and thermal stability. ZIF-8 consists of zinc clusters linked to 2-methylimidazole units, and it's mostly synthetized through the solvothermal method, where ZnX_2 ($X = CH_3COO^-$, Cl^-, NO_3^-) and 2-methylimidazole are separately dissolved in an appropriate solvent and consequently mixed. The traditional solvent for this reaction is *N*,*N*-Dimethylformamide (DMF), which provides both high yield and great crystallinity of ZIF-8 [2]. The current route based on DMF employment accounts for the SCENARIO 1 of our LCA. However, DMF is a fossil-based solvent, hazardous for the environment and potentially toxic for humans. Consequently, the development of new sustainable synthetic strategies represents an urgent requirement to be satisfied. In this regard, glycerol carbonate (GlyC) has been successfully tested for the first time [3] as a green solvent for the synthesis of ZIF-8, showing comparable performances to DMF in terms of both yield and crystallinity of the products. Reaction mixture recycle has also been tested for subsequential reaction cycles, showing the ability to carry out ZIF-8 synthesis after five consecutive reactions. As a result, this innovative pathway represents the SCENARIO 2 of our LCA. A comparative LCA between scenario A and scenario B is comprehensively described, highlighting the "hotspots" for each process, from the supplying of the reagents (cradle) to the final product obtained at a laboratory scale (gate).

Keywords: *Life Cycle Assessment, cradle to gate, ZIF-8, glycerol carbonate*

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Experimental and Theoretical Insights into a Novel Lightfast Thiophene Azo Dye

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Thiophene ring-enhancing electron delocalization imparts unique properties to azoic chromophore tools. The novel TA-OH dye contains a push–pull π-electron system, including a thiophene-azo scaffold with a hydroxyl group at the ortho position to the azo bridge. The hydroxyl group is expected to lock the azo bridge in its trans conformation, concurring with the photostability and fastness of the dye. The single crystal analysis identified the molecule's primary conjugation plane, and the theoretical analysis provided electronic pattern insights. The absorption behavior and the trans-to-cis conversion were examined from both experimental and theoretical perspectives. The effect of solvent polarity and the role of pH on the photophysical properties were explored. The solvent polarity strongly affects the absorbance spectrum of TA-OH, therefore potentially making NLO active. Additionally, TA-OH exhibited pH responsiveness akin to classic dichromatic pH indicators, with a noticeable color shift from red to blue observed as pH transitioned from neutral to alkaline. Absorbance titration experiments, along with experimental/theoretical determination of pKa, defined the pH sensing ability.

Keywords: *azo dyes; thiophene; theoretical insight; pH-responsive; lightfastness**
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NMR-based profiling isolation and structural elucidation of potentially bioactive oleanane saponins from *Bellis sylvestris Cyr.*

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Saponins are specialized metabolites exerting a wide range of unique biological properties. In the recent past, there has been unforeseen interest in the clinical utilization of saponins as chemotherapeutic agents. The profound impact of saponins on cancer cells has gained significant research interest in the pharmaceutical sector[1], since these compounds have demonstrated outstanding potential in inhibiting different cancer cells under in vitro and in vivo conditions. The current work was addressed to the isolation and structural characterization of new potentially bioactive oleanane saponins from *Bellis sylvestris Cyr.*, applying a metabolomic approach based on a combination of high-resolution spectroscopic techniques and phytochemical studies[2]. The NMR-based profiling allowed to analyze the formerly purified leaf fractions of *B. sylvestris* that showed a considerable cell growth inhibitory activity, revealing the presence of new oleanane saponins. These saponins, isolated through chromatography, shared a similar aglyconic skeleton with previously identified compounds3. However, 2D NMR analyses proved significant differences, allowing a structural elucidation of aglycone and sugars. Interestingly, the NMR-based profiling also revealed the presence of new saponins in the flower heads and roots. Assuming their structural resemblance to those isolated from the leaves, a phytochemical investigation of the roots extracts was implemented. The study, through chromatographic procedures, led to a complex mixture of saponins. NMR analyses of isolated pure saponins within indicated a same core structure, different in term of degree and position of acetylation sites on the oligosaccharide fraction esterified to C-28. The hypothesis of a shared monodesmoside structure was substantiated through a complete basic hydrolysis. Subsequently, a partial alkaline hydrolysis proved the presence of a common sugar chain linked to C-28 among the saponins in the mixture. Then, extensive 2D NMR analyses of the purified partial hydrolysis product not only confirmed the aglycone structure, but also allowed the structural elucidation of the oligosaccharide chain esterified to C-28. Subsequent studies will aim to evaluate the anticancer efficacy of these new potentially bioactive pure compounds, seen the promising results obtained from the bioactivity screening of the saponin-enriched fraction.

Keywords: *oleanane saponins, NMR*

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Optimization of a Nanoparticles Protein Corona Isolation and Identification Platform using Omics

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Since the engagement of nanoparticles is becoming more and more relevant in modern medicine, scientific attention has been drawn toward the characterization of the "bio-molecular corona", which is described as a layer of biomolecules assembling on the surface of nanoparticles when they encounter biological fluids.
The dynamic structure of the corona is composed of different biomolecules, such as lipids, sugars, nucleic acids and, first and foremost, proteins accounting for most of the overall mass. Therefore, we mostly refer to this entity as "protein corona".[1]
The "protein corona" provides a new identity to the nanoparticles, hence redefining their pharmacokinetics and fate in the body ranging from their uptake by cells to immunologic responses and resulting in a decrease in their half-life. Therefore, the "protein corona" characterization is essential in the development of nanoparticles with better bioavailability, but it can also help in resolving the nanoparticles path inside the body, opening the way to a targeted delivery of therapeutics.[2]
One of the main challenges in the corona characterization is the optimization of its isolation from the biological environment. In literature, centrifugation is the most frequently described technique since it can be applied to the vast majority of nanoparticle types even though many drawbacks are evident, such as potential disruption of the physiological corona or the creation of artefacts caused by the applied centrifugal force.[3]
Thus, with the aim of an in-depth protein corona characterization, we set to establish the best strategy for corona analysis using polylactic-co-glycolic acid (PLGA) nanoparticles as a model. In particular, preliminary experiments were performed to define the optimal isolation method for the nanoparticle-corona complexes, starting from centrifugation and moving on to less common methodologies, such as microfiltration and size exclusion chromatography, also implementing an array of different variables for the biological fluids.

Keywords: Proteomics, Nanoparticles, Corona, Mass Spectrometry

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Lanthionine determination in serum of patients affected by Chronic Kidney Disease by Multiple Reaction Monitoring

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CKD is defined as a persisting abnormality in function or structure of kidney of at least 3 months. There are many observed parameters such as: GFR less than 60 ml/min; albuminuria, urine sediment; a kidney damage; renal tubular disorders or kidney transplantation. CKD is also related to accumulation of uremic toxins in serum, such as lanthionine, homocysteine, homolanthionine, cystathionine. Lanthionine is a nonproteinogenic amino acid and is a side product of H_2S production. For this reason, it has been proposed as a marker of H_2S synthesis. This is a preliminary study that will be part of a larger project, in which CKD will be related to cognitive disorders, for instance deficits in memory, attention, language, and visuospatial skills. HPLC-MS/MS in Multiple Reaction Monitoring modality will be analytical methodology applied for the determination of Lanthionine, but also a large class of water-soluble molecules, middle molecules (for example beta-2-microglubuline, interleukins) and protein-bound compounds. The first analysis performed, showed different levels of lanthionine, related to different stages of CKD. Particularly a gradual increment of lanthionine from stage 1 to 5.

CKD, Uremic toxins, tandem mass spectrometry

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Derivation of Nuclear Magnetic Shielding and Magnetizability in Open-Shell Systems Throughout a Semi-Relativistic Approach.

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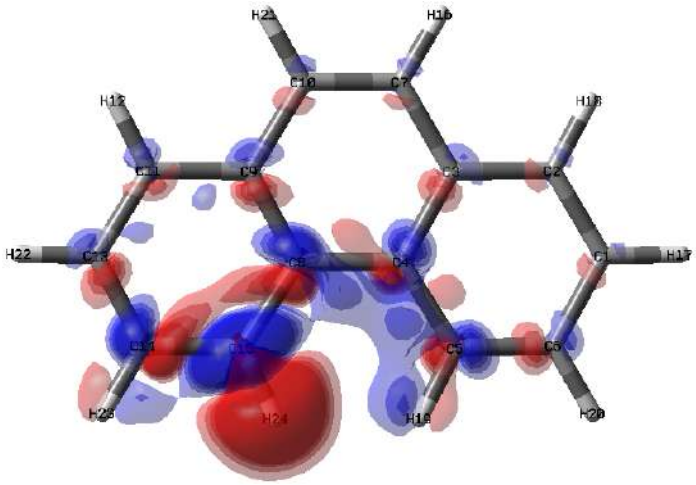
The interaction of matter with electric and magnetic fields or both has always fascinated the scientific community. Particularly in the case of open shell systems, NMR and EPR spectroscopies are useful means to identify their structure being sometimes the existence of these species very short. There is a close connection between these spectroscopies and the induced current density in molecular systems, so it seems useful to elucidate the connections of this vector field with the observed magnetic properties, like nuclear magnetic shieldings and magnetizabilities. It is found that only the spin contribution to these properties depends on the temperature.



Proton magnetic shielding density function of H24 in phenanthrene molecule evaluated at B3LYP/6-31G(d) level of theory. Red and light blue isosurfaces values are ± 0.004, ± 0.003, ± 0.002, ± 0.001 a.u., which include shielding/deshielding regions.

Keywords*: spin currents, spin-orbit coupling, magnetic properties*

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Ethyl levulinate ketalization with glycerol: from batch to continuous operation

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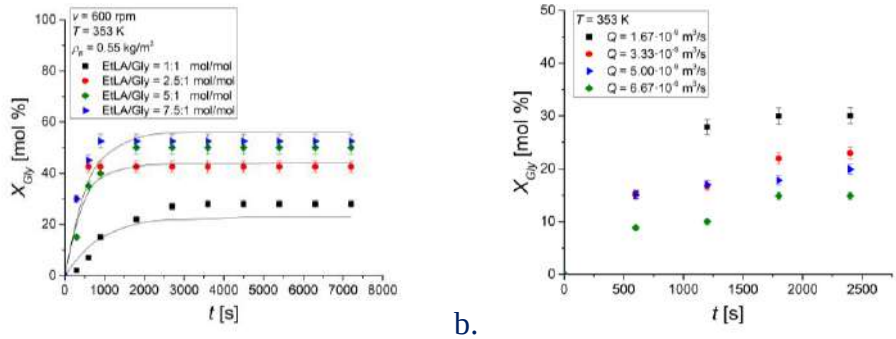
Levulinic acid ketals are considered of great interest thanks to their proprieties such as high solvency and compatibility with several applications as lubricants, plasticizers and solvents [1]. They can be obtained from the reaction between levulinic acid itself or its esters with polyols promoted by acid catalysts. Traditionally, the reaction has been carried out with homogeneous Brønsted acid catalysts, but their corrosive action has led to search for more suitable systems. Therefore, heterogeneous catalysts have acquired increasing interest, as ion exchange resins and zeolites [2]. The choice of ethyl levulinate among the possible esters of levulinic acid is due to the advantages connected with its use, such as the possibility of being obtained from the reaction of biomass derived raw materials with ethanol. In this study, a kinetic investigation of ethyl levulinate (EtLA) ketalization with glycerol (Gly) was conducted in the presence of H,Y-zeolite. The reaction was performed in both batch reactor and continuous milli reactor, employed in order to reduce eventual liquid-solid mass transfer limitations, that could occur using classical packed bed reactors.



a. b.

Figure 1. a. Reactants molar ratio effect for tests made in batch. b. Volumetric flow effect for tests made in the milli-reactor.

The effect of different operating conditions on the reaction rate was examined, and a kinetic model was developed for both devices to interpret experimental data and estimate kinetic and thermodynamic parameters.

Keywords: *Ethyl levulinate, glycerol, ketalization, kinetics, milli-reactor*

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Biosurfactant for eco-sustainable formulations: rhamnolipids as multifunctional component

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There is a growing interest in industrial formulations based on natural ingredients that have a low environmental impact [1]. Biosurfactants derived from fungi, yeasts or bacteria is a key strategy to obtain products that are biocompatible and biodegradable. In particular, rhamnolipids appear to be suitable candidates for replacing synthetic surfactants [2]. They are biotechnologically produced from industrial waste oils, are safe, biocompatible, biodegradable and available at low cost [3]. Although the micellization of rhamnolipids in dilute solutions is well known, their self-aggregation in concentrated mixtures remains largely unexplored.
In this contribution, Electron Paramagnetic Resonance (EPR) spectroscopy is used to study the local structure and dynamics of concentrated aqueous rhamnolipid mixtures using paramagnetic molecular probes. A detailed computational analysis of the EPR spectral components shows a dramatic molecular reorganization of the mixture starting from 80 wt% rhamnolipid, suggest more compact lipid aggregates. These observations suggest a phase transition that is currently being investigated by SAXS for meso-morphological characterization of aggregates. Preliminary results indicate the presence of unstructured ordered phases [4]. The concentrated rhamnolipid mixtures are currently also being studied by rheology, as viscoelastic properties are fundamental for a variety of practical applications [5]. Concentrated samples exhibit shear thinning at 45 °C, suggesting a non-isotropic solution. As the temperature decreases, shear thinning is not observed at 70%, while it remains present at high concentration.
The presence of endogenous free radicals in the samples is revealed by EPR and correlated with the antioxidant activity of the rhamnolipids. The persistent signal registered even in the absence of spin probes suggests the formation of meso structures capable of stabilizing radical species, thus acting as "redox buffers". Our results highlight rhamnolipids as promising multifunctional components for eco-sustainable formulations where, in addition to the typical role of surfactants (e.g. foaming or emulsifying agents), they can also act as pro- or anti-oxidants depending on the external conditions.

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Keywords: *bio-surfactanct, Electron Paramagnetic Resonance, formulations.*

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Engineered silica nanoparticles for wastewater remediation

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In response to the increasing efforts to preserve water sources, there has been a significant rise in the development of technologies for collecting, recovering, and preserving wastewater. In this field, integrating nanotechnology into pollution recovery systems represents a significant step toward achieving this important objective [1].
Silica nanoparticles (SiO2-NPs) are highly promising for treating inorganic contaminants due to their unique properties, such as high surface area and high adsorption capacity. Furthermore, SiO2-NPs can be easily synthesised and functionalized in an appropriate way by sol-gel synthesis, in order to extend their applicability to organic contaminants like dyes [2-3].
This work illustrates sustainable approaches for the design and development of silica nanoparticles that can be used to wastewater remediation by adsorbing contaminants from various sources. Adsorption efficiency was investigated in regard to methylene blue, 4-nitrophenol, and heavy metal ions. The results demonstrated how the final adsorption efficiency of SiO2-NPs are significantly influenced by particles architectures.

Keywords: *sol-gel synthesis, silica nanoparticles, water remediation*

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NMR-based strategies for the rapid identification and characterization of anticancer specialized metabolites from Mediterranean Asteraceae species

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Despite medical and clinical advancements, due to the development of resistance to therapies currently employed in the clinical setting, cancer remains a serious chronic disease with a rising prevalence, underscoring the need for new anticancer treatments [1].
In this context, natural products from plants and their derivatives are promising therapeutic agents due to their unique structural characteristics[2].
Here, we report an accurate characterization of twelve Asteraceae plant species allowing the identification of two promising leaf extracts (*Centaurea deusta* Ten. subsp. *deusta* and *Xanthium italicum* Moretti) through combination of NMR-based metabolomics profiling and biological activity analysis. In particular, to accelerate the discovery and characterization of potential biomolecules, innovative NMR pulse sequences were applied to crude extracts. The key spectral regions identified by NMR-based metabolomics were further analyzed using advanced selective NMR experiments (PSYCHE, PS-*ed*HSQC, *sel*-TOCSY, DOSY), leading to the rapid identification of five sesquiterpene lactones[4]. In-mixture NMR experiments facilitated the development of a specialized phytochemical strategy (L-L extension, CC, HPLC), avoiding traditional bio-guided fractionation, and reducing costs and time.
This process resulted in the isolation of four xanthanolide and one germacranolide sesquiterpene lactones, whose structures we successively confirmed by multi-dimension NMR techniques (COSY, HSQC, H2BC, HSQC-TOCSY, CIGAR-HMBC, TOCSY, and NOESY).
Additionally, preliminary *in vitro* studies demonstrated the analyzed extracts present significant biological effects on cell proliferation and cell death induction, consistent with the observed substitution pattern.

Keywords: *NMR-based metabolomics, specialized metabolites, cancer, drug discovery*

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Exploring Equilibria between Monomeric and Oligomeric species involved in Prion diseases

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Prion diseases are rare and fatal neurodegenerative disorders, characterized by the deposition of fibrillary protein aggregates. It has been recognized that soluble oligomers which form during fibril assembly could be the key cytotoxic species associated with cellular dysfunction and disease onset [1]. These oligomers are often lowly-populated, rapidly interconverting, and transiently formed, making them challenging targets for many biophysical approaches, however Nuclear Magnetic Resonance (NMR) techniques have the ability to quantitatively probe alternative states or excited states in dynamic equilibrium with the dominant ground conformation [2]. The aim of this study is to provide a high-resolution description of the molecular determinates driving the early stages of the amyloids assembly pathway initiated by a stable β-enriched intermediate state (β-PrPI) of truncated HuPrP (90-231) [3]. To address this purpose we applied Chemical Exchange Saturation Transfer (CEST) NMR experiments [4] to obtain a detailed description of kinetic and thermodynamic parameters related to human prion conformational equilibria involving transient oligomeric species that drive amyloid fibril assembly mechanism. In the absence of oligomeric species, ^{15}N CEST data acquired at low temperature (15°C) did not show any detectable conformational exchange. On the contrary, in the presence of β-PrPI-oligomers, ^{15}N CEST experiments revealed that the HuPrp(90-231) monomeric state is in slow conformational exchange with "NMR-invisible" oligomers, having a pronounced β-strand character. Interestingly, ^{15}N chemical shift values of β-PrPI-oligomers are aligned with the Cryo-EM structure of prion fibrils [5], providing insights about structural rearrangement involving HuPrP(90-231) N-term tail.

Keywords: *Solution NMR spectroscopy, prion protein, chemical exchange saturation transfer*

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Il Contributo dei Giovani Chimici in Campania
Edizione 2024

NOVEL SULFONATED N-HETEROCYCLIC CARBENE SILVER(I) AND GOLD(I) COMPLEXES IN A^3-COUPLING CATALYSIS

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Alkynes are versatile organic compounds due to their unsaturated bond which are well suited for oxidative processes and electrophilic additions. Alkynes can serve as both nucleophilic and electrophilic groups, with the latter being harder to handle without the aid of metallic salts or organometallic complexes[1]. Transition metals such as silver and gold have a strong affinity for alkynes. Acting as soft Lewis acid centers, they can coordinate unsaturated bonds, facilitating nucleophilic addition and the formation of acetylides in mild conditions.[2,3] The search for better ligand classes in these complexes is ongoing because of their applicability, and N-heterocyclic carbene (NHC) ligands are of particular interest[4]. NHC ligands were first synthesized and characterized as stable carbenes by Arduengo[5]. These ligands are renowned for their versatility and ability to stabilize organometallic structures. As a result, Ag(I)- and Au(I)-NHC complexes have found wide applications in catalysis with alkyne substrates to promote attractive and more environmentally sustainable reactions. Our research aims to synthesize, characterize, and use novel homoleptic sulfonated N-heterocyclic carbene silver(I) and gold(I) complexes in A^3-coupling reaction (alkynes, aldehydes, amines).[6,7]

Keywords: *Ag(I)-NHC, Au(I)-NHC, alkynes functionalization, homogeneous catalysis*

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Production of hybrid lipid/polymer nanoplatforms for RNA delivery by an emulsion-solvent diffusion technique: from benchtop to microfluidics

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Our research group has developed and validated a panel of inhalable hybrid lipid/polymer nanoparticles (hNPs) for siRNA delivery to the lungs produced by emulsion solvent diffusion technique [1,2]. The hNPs have demonstrated their potential in several *in vitro* models[1,2], while *in vivo* validation is currently underway. As a viable alternative to conventional emulsion techniques, microfluidic is revolutionizing nanoparticle (NP) production, providing for precise control over NP features, such as size and morphology, at the same time assuring good reproducibility[3]. Along these lines, the aim of this project is adapting the benchtop protocol for RNA-loaded hNP production to a microfluidic system. To achieve this goal, we employed the Automated Nanoparticle System (Particle Works), a microfluidic device able to produce monodisperse nanoparticles with high efficiency. Various manufacturing conditions were tested, including flow rate, canal shape, and material concentrations, to produce hNPs consisting of a poly(lactic-co-glycolic) acid (PLGA) core and a dipalmitoyl phosphatidylcholine (DPPC) shell. Polyvinyl alcohol (PVA) was added as stabilizer. Special attention was paid to the process parameters to obtain hNPs able to meet the quality attributes needed to successfully exploit RNA delivery through the pulmonary administration route. All formulations were characterized for hydrodynamic diameter (D_H), polydispersity index (PDI), and zeta potential (ζ). Particle size distribution and concentration were confirmed by the Nanosight Pro (NTA) system (Malvern Panalytical). All the sample were observed by Transmission Electron Microscopy (TEM) to evaluate the hNPs morphology. Additionally, thermodynamic analysis through Differential Scanning Calorimetry (DSC) was carried out. The optimized hNPs were further tested for their ability to encapsulate model siRNA. Under controlled conditions, the hNPs produced by the ANP System exhibited a spherical morphology with a D_H smaller than 200 nm, a PDI of approximately 0.150, and a negative ζ potential (~ -30 mV). Thermodynamic studies further validated the structural integrity of the hNPs. Preliminary studies have showed the ability of the nanosystem to encapsulate model siRNA sequences. The overall characterization of hNPs produced using the ANP microfluidic system supports the successful transition of the emulsion solvent diffusion technique from a lab-scale to a microfluidic platform without compromising the critical quality attributes of hNPs. Studies are ongoing to confirm the tolerability and therapeutic efficacy of the developed hNPs in cell models of lung fibrosis.

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Keywords: *manufacturing, microfluidics, benchtop, Hybrid lipid polymer nanoparticles, RNAs.*

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Thermosensitive in situ gelling poloxamers/hyaluronic acid gels for hydrocortisone ocular delivery

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Ocular drug delivery is limited by several obstacles, including rapid elimination and enzymatic metabolism which cause low bioavailability and the need for frequent dosing, resulting in potential adverse effects [1]. Thermosensitive in situ gelling ophthalmic systems, made up of heat-sensitive amphiphilic block copolymers, such as poloxamers, may address these issues. Poloxamers are triblock copolymers, composed of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO-PPO-PEO), which undergo self-assembly through micellization and may offer extended precorneal permanence compared to conventional ophthalmic drug delivery systems [2]. Nevertheless, poloxamer-based gels exhibit some limitations, such as low stability, poor mechanical properties, and short residence times. These issues may be overcome by blending them with hyaluronic acid (HA) that enhances mucoadhesive and mechanical properties of the platforms [3-4].
Thus, in this study we designed and produced different thermosensitive gels made up of Poloxamers and HA for ocular hydrocortisone (HC) delivery. The gels were characterized for their viscoelastic properties, gelation temperature/time, morphology and physicochemical characteristics. Release kinetics of HC from the gels were assessed by spectrophotometric assay. Only three platforms were selected based on their gelling ability and time, namely P3/0.1, P3/1 and P4/0.1, containing 21.43 or 30% w/V overall poloxamers concentration and 0.1 or 1% w/V of HA. SEM micrographs and FTIR spectra reveal the presence of a distinct network structure, characterized by an ordered arrangement of poloxamer micelles leading to hydrogel formation. Thermal analysis indicate that HA incorporation hampers the interactions between water molecules and poloxamers. HC release is faster when HA content increases from P3/0.1 to P3/1 formulations. HC release rate from P4/0.1 gels is intermediate between P3 formulations, probably because the higher poloxamers concentration allows an intimate blending with HA and, hence an overall higher hydrophilicity. In all cases, an initial fast release phase was observed, followed by a slower one.
The produced platforms offer promising prospects for efficacious ocular drug delivery, addressing pivotal challenges in ocular therapeutics and heralding future advancements in the domain.

Keywords: *Ophthalmic drug delivery; hyaluronic acid; hydrogels; Thermosensitive systems; Hydrocortisone release kinetics.*

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HPβCD Impact on Zein Edible Films for Food Packaging

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In recent years, environmental, ecological, and safety concerns over petrochemical-based, non-biodegradable packaging materials have increased the emphasis on the use of biodegradable alternatives [1]. Compostable and renewable materials can be manufactured from lipids, polysaccharides, and proteins, including prolamins [2]. Among these, zein, a biodegradable corn prolamin, due to its physicochemical properties, stands out as a suitable candidate for the development of edible packaging applications used in food storage. However, the brittle nature of zein films limits its application as a universal packaging material [3]. Therefore, this study aims to develop an innovative packaging system composed of zein, 2-hydroxypropyl-beta-cyclodextrin (HPβCD), and Poly(ethylene glycol) (PEG 400) as a plasticizer.
Initially, we investigated zein solubility in different media (ethanol 80% and 90% v/v) at varying concentration and temperature through transmittance and dynamic light scattering measurements (DLS). The transmittance values of the samples decrease with lower temperatures, reduced ethanol content, and higher zein concentrations. Coherently, in these same circumstances, an increase in particle size is observed. Upon the addition of HPβCD to zein dispersions, an increase of transmittance and a more reproducible size distribution curves were found, indicating a reduction of zein aggregation state. These results clearly indicate that HPβCD affect zein aggregation by interacting with single amino acids and intrachain interactions.
Then, zein films were prepared by casting at 50°C zein and zein/HPβCD dispersions, formulated from a 90% v/v ethanol solution at 70°C. All the systems underwent comprehensive characterization including scanning electron microscopy (SEM), mechanical properties analysis, water vapor permeability (WVP), moisture content (MC), water solubility (WS), optical barrier properties evaluation and antioxidant activity measurement. Results suggest that HPβCD held a crucial role in the formulation process providing films with optimized WVP, WS and MC values, improved mechanical properties and morphological uniformity and enhanced UV-blocking ability which are crucial parameters for the performance and stability of the final packaging system.
Although HPβCD has not yet received approval for food applications, primarily due to cost rather than safety concerns, the zein/HPβCD platforms developed can be considered a promising substitute in the realm of innovative biodegradable packaging, offering a sustainable alternative to traditional materials.

Keywords: *zein, HPβCD, edible films, dispersion*

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Highly efficient synthesis of zeolitic imidazolate framework-8

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Zeolitic imidazolate frameworks (ZIFs) are a subclass of the well-known metal organic frameworks (MOFs), a new kind of crystalline porous materials consisting of metal cations coordinating organic ligands. These materials have gained an increasing interest among the scientific community for their extensive structural design possibilities. Among them, ZIF-8 stands out, consisting of tetrahedral units where each Zn ion coordinates four 2-methylimidazolate linkers, forming a three-dimensional sodalite-type topology. Due to their remarkable properties such as high crystallinity and porosity, large surface area and exceptional thermal and chemical stability, ZIF-8 are considered as attractive candidates for various applications including catalysis, gas storage and separation, drug delivery and sensing application. The properties of ZIF-8 are strongly influenced by synthesis methods and parameters like temperature, pH, reaction time, solvent, reactants' molar ratio and concentration. In this respect, many efforts have been made to control the structural and morphological properties of ZIF-8 through the synthetic process. The solvothermal method using dimethylformamide (DMF) as solvent is commonly employed for the traditional synthesis of ZIF-8 [1]. However, since DMF is a toxic, fossil-based solvent that poses risks to human health and to the environment, developing new synthetic processes that use alternative solvents has become a primary goal in this field. In this context, glycerol carbonate (GlyC) has been successfully tested for the first time [2] as a green solvent for the synthesis of ZIF-8, showing a comparable performance to DMF but with lower environmental impacts. Nevertheless, the high solvent volumes required still represent a challenge. To overcome this limitation, this work aims to explore the valorization of the waste obtained as by-product from the synthesis of GlyC, specifically the dimethyl carbonate-methanol mixture (DMC-MeOH), as an alternative solvent for the synthesis of ZIF-8. The latter involves $Zn(OAc)_2$ and 2-methylimidazole (Hmim) as precursors, in the presence of NaOH. Additionally, the synthesis of ZIF-8 is investigated using pure DMC, a highly recommended green solvent due to its non-toxicity and biodegradability (90% within 28 days) [3]. Various experimental conditions and synthesis parameters are being investigated to optimize the new proposed processes, in particular different reactants' concentrations as well as different temperatures, while keeping reaction time and reactants' molar ratio fixed. Finally, following the Green Chemistry principles, the potential of recycling the used solvents for further ZIF-8 synthesis is being evaluated to minimize the environmental impacts related to the process. Green metrics like E-factor and Process Mass Intensity (PMI) are being determined to quantify respectively the waste and the mass effectiveness of the reaction.

Keywords: *zeolitic imidazolate framework (ZIF), porous materials, synthesis*

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Quantitative analysis of self-diffusion coefficients (D_t): a semi-empirical model for aqueous solutions

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Measuring translational self-diffusion coefficients (D_t) in solution is becoming a routine in modern chemical laboratories, owing to the advancements in electrochemistry and diffusion NMR spectroscopy. Quantitative analysis of translational self-diffusion coefficients (D_t) is a powerful tool, for instance, to estimate the molecular size of an analyte, which however requires careful calibrations and data manipulation [1]:
In organic solutions, quantitative analysis of D_t is non-trivial but possible. A modified Stokes-Einstein equation, correlating the D_t with the hydrodynamic radius of the studied molecule (r_H), is typically used:

$$D_t = \frac{k_B T}{c f_s \pi \eta r_H} \quad (1)$$

where k_B is the Boltzmann constant, T the absolute temperature and η the fluid viscosity, while f_s and c are two correction factors respectively accounting for the shape of the analyte and its relative size compared to the solvent [2]. The main difficulty in this approach lies in the estimation of the c parameter, since it is itself a function of r_H. Several models have been proposed to address this problem, with the one reported by Chen and Chen being the most effective and widely used [3]:

$$c = \frac{6}{1 + 0.695\left(\frac{r_{solv.}}{r_H}\right)^{2.234}} \quad (2)$$

Unfortunately, this approach fails in aqueous systems because, while Eq. 1 is a general law based on first principles, Eq. 2 is derived semi-empirically and specific for organic solvents.
Recently, we have identified a suitable strategy to adapt the Chen and Chen model for estimation of c to aqueous system [4]. This approach accounts for the peculiarities of a "structured" solvent like water, particularly with respect to its ability to establish a network of strong hydrogen bonds with itself and polar solutes. This novel semi-empirical model will be presented, highlighting its potentialities for the analysis of small molecules, supramolecular aggregates, and biological systems in water.

Keywords: *Diffusion coefficients, hydrodynamic volume, hydrogen bonding, aqueous solutions*

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