UNIVERSITÀ DI PARMA

Società Chimica Italiana
Gruppo Interdivisionale Biotecnologie

Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale

4° Workshop

I chimici per le biotecnologie

Venerdì 1° Luglio 2022-Ore 10.30-17.30
Aula Congressi del Plesso Aule delle Scienze
Università degli Studi di Parma, Campus Scienze e Tecnologie
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4° Workshop "**I CHIMICI PER LE BIOTECNOLOGIE"**
Parma, 1 Luglio 2022

PROGRAMMA

10:00	10:30	**Accoglienza partecipanti**		
10:30	10:45	**Apertura lavori e saluti di benvenuto** Prof. Laura Cipolla – Coordinatrice CIB Prof. Paolo Martelli – Prorettore vicario dell'Università degli Studi di Parma Prof. Maria Valeria D'Auria – Presidente della Divisione di Chimica Organica della SCI Prof. R. Corradini – Direttore Dip. di Scienze Chimiche, della Vita e della Sostenibilità Ambientale		
10:45	11:05	**Invited talk**		*Chair: Prof. O. Piccolo/L. Cipolla*
IT1 **Massimo Rastelli**			GlaxoSmithKline	*The future grows with the molecular weight – Evolution in the pharmaceutical industry*
11:05	11:35	**Oral Communications**		
11:05	11:15	**OC1** **Maria Maddalena Calabretta**	Alma Mater Studiorum - Università di Bologna	*Towards cancer avatars with bioluminescent microtissues*
11:15	11:25	**OC2** **Giovanna Viola**	Università degli Studi di Verona	*Targeting the amyloidogenic protein tau with ultrasmall gold nanoparticles*
11:25	11:35	**OC3** **Diego Tesauro**	Università degli Studi di Napoli Federico II	*Reactivity of S- or Se- containing model peptides with environmental relevant Hg ions: LC-MS/MS study*
11:35	12:00	**Flash Communications**		*Chair: Prof. R. Corradini*
11:35	11:40	**FC1** **Matteo Calvaresi**	Alma Mater Studiorum - Università di Bologna	*Orthogonal nanoarchitectonics approach to engineer M13 bacteriophages as theranostic targeted platforms*
11:40	11:45	**FC2** **Carlo Alberto Vezzoni**	Università degli Studi di Parma	*An argininocalix[4]arene for the effective delivery of miRNAs*
11:45	11:50	**FC3** **Lara Grenzi**	Università degli Studi di Modena e Reggio Emilia	*Optimizing Novel Cholesterol Nanomedicines for Brain Delivery*
11:50	11:55	**FC4** **Sara Messori**	Università degli Studi di Modena e Reggio Emilia	*Microfluidic technology optimization for the formulation of peptide-loaded liposomes*

11:55	12:00	**FC5 Matteo Di Glosia**	Alma Mater Studiorum - Università di Bologna	*Proteins as Trojan Horse for biomedical applications of fullerenes*
12:00	12:20	**Invited talk**		*Chair: Prof. L. Cipolla*
		IT2 Andrea Tortori	Bioridis	*New approaches for the analysis of Nucleic Acids*
12:20	12:50	**Flash Communications**		*Chair: Prof. L. Cipolla*
12:20	12:25	**FC6 Francesco Secundo**	Istituto di Scienze e Tecnonolgie Chimiche "Giulio Natta", CNR, Milano	*Whole-cell biotransformation of 3-chloropropiophenone by microalgae Chlorella emersonii entrapped in hydrogel*
12:25	12:30	**FC7 Valeria Pappalardo**	SCITEC CNR, Milano	*Catalytic lipophilization of natural antioxidants*
12:30	12:35	**FC8 Francesco Presini**	Università degli Studi di Ferrara	*Biocatalytic process for the production of enantiomerically enriched vicinal diols: from in vitro to in vivo system*
12:35	12:40	**FC9 Federico Acciaretti**	Università degli Studi di Milano-Bicocca	*From dairy waste to hexose-derived building blocks*
12:40	12:45	**FC10 Francesca Frongia**	Università degli Studi di Modena e Reggio Emilia	*Microalgae potential in the capture of carbon dioxide emission*
12:45	12:50	**FC11 Gianmarco D'Ambrosio**	Università degli Studi di Parma	*Evaluation of the effects of fermentation on carbohydrate profile in low-FODMAP baking products*
12:50	13:55	**Pausa pranzo**		
13:55	14:15	**Invited talk**		*Chair: Prof. G. Oliviero*
		IT3 Serena Riela	Università degli Studi di Palermo	*Clay minerals for an interdisciplinary research frontier*
14:15	14:45	**Oral Communications**		
14:15	14:25	**OC4 Federico Verdini**	Università degli Studi di Torino	*Residual lignocellulosic biomasses for sustainable production of PHAs*

14:25	14:35	**OC5** **Edmondo Messinese**	Università degli Studi di Parma	*Agro-food by-products as sustainable resource: analytical approaches for bioactive compounds characterization*
14:35	14:45	**OC6** **Marina Simona Robescu**	Università degli Studi di Pavia	*A two-step enzymatic approach to cheese whey valorization: Synthesis of alkyl galactoside fatty acid esters as non-ionic biosurfactants*
14:45	15:05	**Invited talk**		*Chair: Prof. A. Romanelli*
IT4 **Loredana De Bartolo**			CNR-ITM, Università della Calabria	*Advances in membrane systems for biofabrication of tissues and organs*
15:05	15:45	**Oral Communications**		
15:05	15:15	**OC7** **Laura Legnani**	Università degli Studi di Milano-Bicocca	*Computational Mechanistic study of Ruthenium Tetroxide Oxidation of N-Methyl-Isoxazolidine*
15:15	15:25	**OC8** **Valentina Venturi**	Università degli Studi di Ferrara	*Sustainable chemical processes: from the "green practice" of eco-friendly chemistry to a strategy for a successful Company.*
15:25	15:35	**OC9** **Elia Lio**	CNR-SCITEC, Milano; Università degli Studi di Milano	*Green solvent extraction of biomolecules with antimicrobial activity from microalgae*
15:35	15:45	**OC10** **Nicola Di Fidio**	Università di Pisa	*Green valorisation of defatted waste of Cynara cardunculus L. to single cell oil and high-quality lignin*
15:45	16:30	**Flash Communications**		*Chair: Prof. M. P. Costi*
15:45	15:50	**FC12** **Carlo Diaferia**	Università degli Studi di Napoli Federico II	*Peptide-based hydrogels for biotechnological applications: tunable and multivalent matrices for tissue engineering*
15:50	15:55	**FC13** **Simona Petroni**	Università degli Studi di Milano-Bicocca	*Modification of polysaccharides for a new generation of renewable plastics.*
15:55	16:00	**FC14** **Elena Togliatti**	Università degli Studi di Parma	*Characterization of poly (butylene-adipate-terephthalate) – calcium-phosphate glass composites: viscoelastic properties and degradation*
16:00	16:05	**FC15** **Francesca Greco**	Università degli studi di Napoli Federico II	*Synthesis and characterization of an antisense PNA to downregulate the PD-L1 protein overexpression in cancr cells*

16:05	16:10	**FC16 Stefano Volpi**	Università degli Studi di Parma	*Peptide nucleic acids – peptide conjugates as tools for targeting miRNA-mediated drug resistance in chronic myeloid leukemia*
16:10	16:15	**FC17 Lorenzo Tagliazucchi**	Università degli Studi di Modena e Reggio Emilia	*Expression, purification, and characterization of Transcriptional Enhancer Associated Domain (hTEAD4), a promising target for anticancer agents*
16:15	16:20	**FC18 Federica Bovio**	Università degli Studi di Milano-Bicocca	*Evaluation of carboxylation degree on osteocalcin*
16:20	16:25	**FC19 Maria Gaetana Moschella**	Università degli Studi di Modena e Reggio Emilia	*Development of a sensitive biochemical tool to assess the expression levels of recombinant ectopic proteins in vitro engineered cellular systems*
16:25	16:30	**FC20 Stefano De Luca**	Università degli Studi di Parma	*Sustainable PBS-based biocomposite for 3D-printing*
16:30	17:30	**Tavola rotonda: Chimica, Biotecnologie e sostenibilità**		
Modera: **M. Cristina Ceresa**, esperta di Sostenibilità, Direttore di Greenplanner e firma del Sole24ore				
		Marco Di Silvestro, Herambiente, Gruppo Hera, Bologna		
		Stefano Sforza, Dip. di Scienze degli Alimenti e del Farmaco, Univ. degli Studi di Parma		
		Mariangela Stoppa, BioFaber Srl		
		Silvia Rapacioli, BiCT srl		
17:30	17:40	**Chiusura lavori**		

The future grows with the molecular weight – Evolution in the pharmaceutical industry

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In the last decade the pharmaceuticals industry had to quickly react to new challenges posed by new diseases such as Covid-19 and others. In this environment it has become key to accelerate the development of innovative drug products that treat new diseases and that face unmet needs. For these reasons, Pharma companies had been pushed to develop more personalised level of treatment in the form of precision medicines.

Development and commercialisation of new drugs is a complex process highly regulated that must fully comply with GMP expectations.

Working in the pharmaceutical industry nowadays presents challenges but at the same time provides exciting opportunities for chemists and biotechnologists.

Product development is done in partnership with the R&D organizations and requires a deep knowledge of mechanism of action of any criticality of the molecules being manufactured and their compatibility with materials used as part of the manufacturing process. Industrialisation from lab-scale to small and then commercial scale and final process qualification is a long journey that poses several challenges to demonstrate that different scales do not impact the quality of the final drug product. Today, acceleration in product development to commercialisation is key to make the products available for our patients.

Quality controls on raw materials, primary packaging components, intermediates and final drug products are key to ensure that the pharmaceuticals products are manufactured with the right level of quality.

GSK Parma site is fully aligned with the trend observed across the Pharma industry.

Important investments had been made in the last 10 years to build a new facility to manufacture a very effective product (small molecule) for the HIV treatment. New investments have been approved recently to build a new facility at the state-of-the-art for the production of biopharmaceuticals drug products in liquid and lyophilised presentation.

In the present lecture these topics will be discussed with examples based on GSK industrial experience.

New approaches for the analysis of Nucleic Acids

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BIORIDIS is a biotech company founded in 2016. Our mission is improving nucleic acid analysis. We aim at providing faster and easier-to-use assays for R&D and diagnostics. BIORIDIS has managed to break the current technological barrier and develop, in the last 6 years of high-risk research, an innovative assay for nucleic acids analysis: LIVELMIA method. The novelty of our method is a new target recognition system based on a specific probes design, which exhibits ideal characteristics to hybridize target nucleic acids more rapidly, with higher affinity and specificity, improving their recognition. LIVELMIA probes are based on modified nucleic acids known as Peptide Nucleic Acids (PNAs) (ref?). For the first assay application (Fig.1), this technology is coupled with common signalling systems and provided in a simple ELISA-like format for an easy implementation in the laboratory workflow. The assay is designed to work directly on crude sample on a common microplate reader, eliminating extraction, purification and amplification.

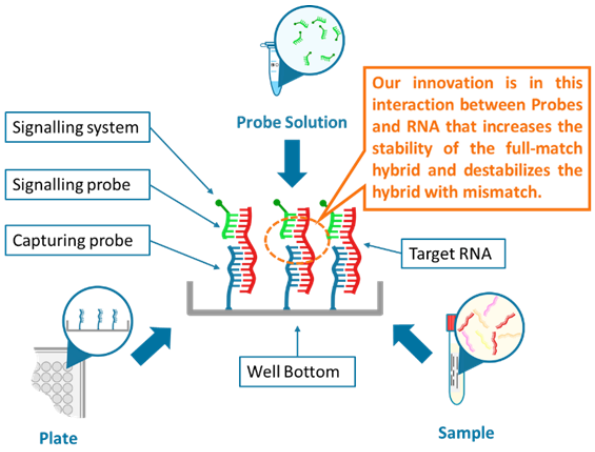

Figure 1: LIVELMIA technology

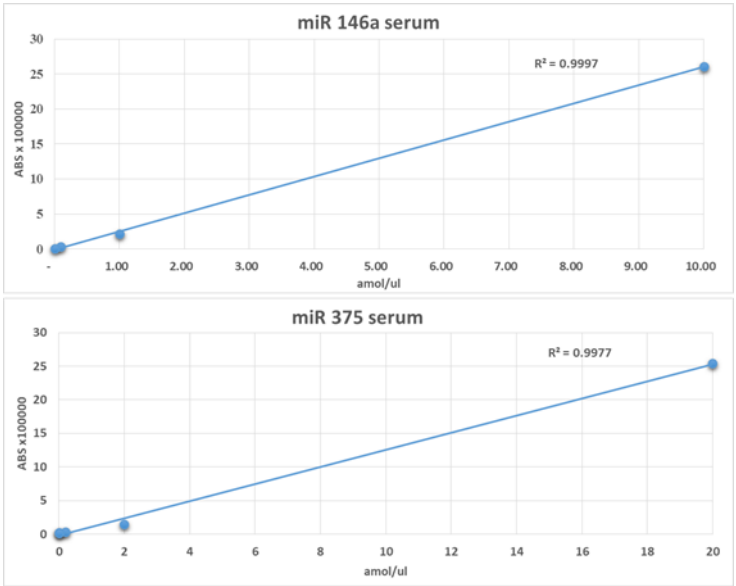

Figure 2: miR-146a and miR-375 standard curve

For the development of LIVELMIA, probes were produced, optimised and tested for certain microRNAs (e.g. miR 146a, 210) and long RNAs such as parts of the SARS-COV2 genome and mRNA of the MecA2 gene of the Methicillin-Resistant Staphylococcus Aureus (MRSA). The assay provides results in less than 90 minutes. The LOD test for miR-146a is 0,01 a(tto)mol/ul (CV between 1 and 5%), standard curve in Fig. 2. Probes design allows for specificity at the level of a single mismatch even in the short sequences of microRNAs. LIVELMIA method has been tested on different biological matrices such as cell lysate, serum, plasma, saliva and urine, with excellent results without the need for preliminary extraction and purification.

With the data collected testing different probes over the years, BIORIDIS developed a software to rapidly design new probes on given target sequencies. For the development of its innovation, BIORIDIS had the support of European grants from both EIT Health and EIC.

Clay Minerals for an Interdisciplinary Research Frontier

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Clay minerals have been used for medical purposes from ancient times. Among them, the halloysite nanotube, an aluminosilicate of the kaolin group, is an emerging nanomaterial which possesses peculiar chemical characteristics. By means of suitable modifications, such as supramolecular functionalization or covalent modifications, it is possible to obtain novel nanomaterials with tunable properties for several applications.[1,2]

Herein it is reported the covalent grafting of suitable organic moieties on the external surface of halloysite to improve the loading and release of several biologically active molecules. The resulting hybrid nanomaterials could be applied as drug carrier and delivery systems, as fillers for hydrogels, in tissue regeneration and in the gene delivery field.

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Advances in membrane systems for biofabrication of tissues and organs

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Tailor-made membranes (e.g., polymeric, composite, functionalized with specific biomolecules) in different configuration can be used for the biofabrication of organs and tissues analogous (e.g., liver, kidney, skin, neuronal system, bone) because of the highly selective properties, which allow to create a fully controlled microenvironment at molecular level.[1] These artificial systems compartmentalize cells in micro- and nano-structured complexes providing a wide surface area for the cell adhesion and ensuring a continuous and selective transport of nutrients and metabolites throughout the cellular compartment while supplying appropriate biomechanical stimuli of the developing tissue. Attempt to engineering biological tissues in vitro have been pursued by applying novel concepts of bioreactor design and membranes to enhance the ability to trigger biological signals that promote the morphogenesis of tissue.[2] This approach allows the realization of microtissues inducing self-assembling process of spheroids through the use of membranes with selective permeability, specific surface and mechanical properties whose design and structural features ensure a uniform microenvironment and adequate oxygenation.[3] A designed approach has been utilized for the development of a 3D hierarchical hepatic tissue based on biodegradable hollow fiber membranes of poly(ε-caprolactone) that compartmentalize human hepatocytes on the external surface and endothelial cells into the lumen of the fibers.[4] A novel membrane bioreactor consisting of poly-L-lactic acid highly aligned microtube array membranes has been created to modulate and enhance neuronal outgrowth, thanks to a synergistic action of the membrane properties and the uniform dynamic bioreactor microenvironment. The membrane bioreactor besides enhancing acquisition of neuronal phenotype guided the neurons into a defined aligned orientation.[5] This platform was used to reproduce an in vitro model of Amyloid beta (Abeta)-induced toxicity associated to Alzheimer's disease to test the neuroprotective effect of molecules such as crocin and glycitein.[6] Polymeric electrospun fibers has been developed for bone morphogenetic protein 2 delivery in bone tissue engineering.[7] The latest developments and innovations regarding the multifunctional role of membrane systems and devices for tissue engineering applications and as in vitro investigational platforms will be discussed.

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Towards cancer avatars with bioluminescent microtissues

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Living cells used as sensing systems have proved to be valuable tools for prediction of the physiological response to drugs, chemicals, and samples in complex matrices, which toxic effects and specific biological activities can be evaluated in an easy and straightforward manner. Thanks to their superior predictivity, 3D cell models (i.e. spheroids, organoids and microtissues) are increasingly replacing conventional 2D cell cultures, enabling to recapitulate the extracellular matrix and cell-cell interactions and creating an architecture that faithfully reflects the native morphology of organs and tumors. Bioluminescent (BL) reporter assays represent the gold standard for several high throughput screening assays employed in drug discovery and BL proteins showed a formidable tool for unravelling molecular pathways involved in the etiopathogenesis of several diseases. A molecular target for cancer studies is p53 protein, considered the "Guardian of the Genome" which protects against the propagation of cells that carry damaged DNA with potentially transforming mutations, activating specific cell death pathways, and metabolic changes in the cell. We hereby report BL microtissues relying on human hepatocellular carcinoma cells (HepG2) genetically engineered with a reporter construct in which the BL protein is placed under the control of the p53 response element. Doxorubicin was selected as chemotherapeutic agent to validate the p53 promoter-based strategy for detecting DNA damage induced by chemicals. Validation of the system is being performed on primary cell lines from a chemically induced hepatocellular carcinoma (HCC) rat model. We envision that this strategy will find broad applications in design and development of avatar cancer models for drug screening and personalized medicine allowing evaluation of drug response, safety, and efficacy in cancer patients with good concordance to the corresponding patient original tumors.

Targeting the amyloidogenic protein tau with ultrasmall gold nanoparticles

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Alzheimer's disease (AD) is the most common form of dementia. It belongs to a group of neurodegenerative diseases, characterised by the deposition of misfolded protein aggregates in specific areas of the brain. The brains of AD patients are characterised by the extracellular accumulation of toxic aggregates of the misfolded amyloid-β and intracellular deposits of the protein tau. [1]

Interfering with protein aggregation has been envisioned as a promising disease-modifying approach for the treatment of AD and other proteinopathies. Nanoparticles (NPs), with their large surface area available for protein adsorption and their small size which facilitates access to tissues and cells, offer significant potential for probing the mechanisms of protein fibrillation and, in the longer term, for treatment of amyloidogenic diseases. [2,3]

Among NPs, ultrasmall NPs (usNPs), usually defined as particles with core size in the range of 1–3 nm, have drawn increasing attention in recent years due to their distinctive physicochemical properties and unique biological behaviours. [4,5]

Moreover, usNP could be used as possible novel therapeutic tools in a great variety of diseases.

In this context, elucidating the interactions of fibrillogenic proteins with NPs and the associated conformational rearrangements could provide the molecular basis for developing new treatments. [6]

In our work, we focused on the synthesis of ultrasmall gold nanoparticles (usGNPs) coated with dihydrolipoic acid, their characterization, and interaction with tau, using various biophysical techniques. [7]

As test protein, we focused on the four-repeat domain of tau (tauK18 or tau4RD) which constitutes the aggregation-prone region of the amyloidogenic tau.

To shed light on the association mechanism between tau and usGNP, we performed photophysical measurements given by the intrinsic fluorescence of both the protein tau and the usGNPs. In addition, we obtained thermodynamics information through isothermal titration calorimetry (ITC). We then studied in detail the protein regions involved in the interaction by site-resolved nuclear magnetic resonance (NMR) experiments. The analysis of NMR spectra showed that the lysine residues are the most involved, underlining the electrostatic contribution of the interaction.

Finally, we explored the activity of usGNP in different aggregation assays. Based on transmission electron microscopy images, we found, that, usGNP influenced aggregation and, at the higher concentration tested, they were able to inhibit the formation of the fibrils.

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Reactivity of S- or Se- containing model peptides with environmental relevant Hg ions: LC-MS/MS study

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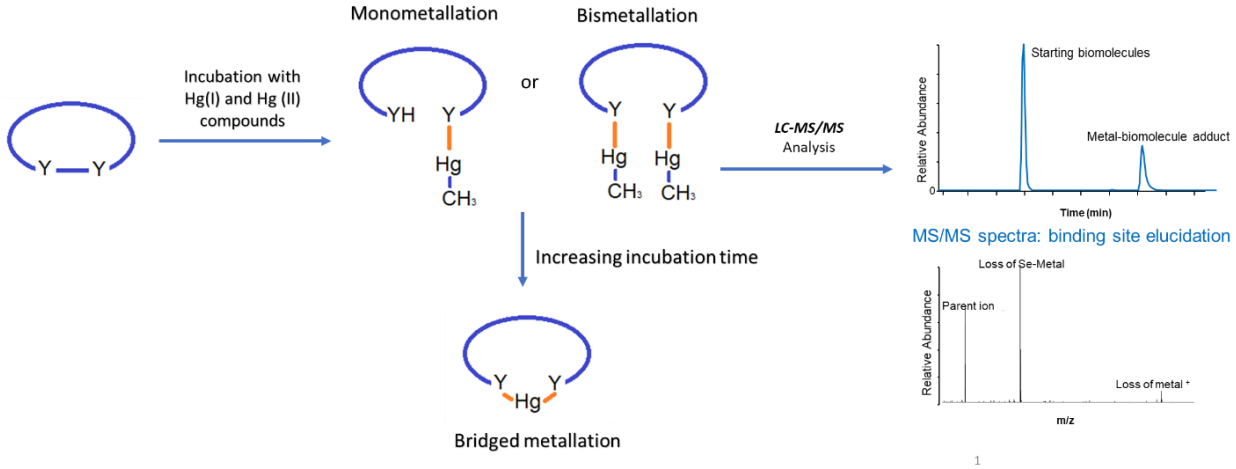
Selenium (Se) is an essential element being present in the form of the naturally occurring amino acid selenocysteine (Sec), 25 human proteins involved in different cellular pathways contain Sec[1]. As the most potent intracellular soft Lewis base, selenocysteine (SeCys) is able to bind electron poor soft acids as heavy metals, of awareness for environmental and human toxicology, Hg ions bind Se by means of higher equilibrium constants than sulfur (ca. 10^6 times), therefore these values compensate the lower cellular abundance (10^5 times) of selenols compared to thiols[2]. In this communication we present a comparative reactivity study of Hg(I) and Hg(II) compounds with model peptides: vasopressin (AVP) hormone with antidiuretic and vasopressor actions and its Sec containing analogs. These peptides were synthesized either by standard solid phase peptide Fmoc or Boc protocols. The metal ion interaction with these peptides was investigated by RP- LC coupled with electrospray MS/MS detection (LC-MS/MS).



We observed mono, bis and bridged peptide metallations as detailed in the Scheme. Taking into consideration the stability of Se-Hg bonds, our results support the hypothesis of a binding preference of Hg to Sec residues in selenoproteins.

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Residual lignocellulosic biomasses for sustainable production of PHAs

Federico Verdini[1], Silvia Tabasso[1], Emanuela Calcio Gaudino[1], Alessio Cirio[1], Francesca Bosco[2], Chiara Mollea[2], Francesco Mariatti[1], Giancarlo Cravotto[1]

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The extensive industrial production of polymeric materials in the 20th century, combined with an inadequate disposal, caused their accumulation in the environment altering biogeochemical cycles and biotic communities [1]. In addition, the intensive exploitation of fossil sources has turned the interest of academia and industry towards the research of alternative polymeric materials derived from renewable sources, such as, for example, polyhydroxyalkanoates (PHA). These biopolymers are biodegradable polyesters of biological origin synthesized by hundreds of bacteria, as intracellular inclusions. Industrially, PHAs are produced by high-cost fermentation processes that exploit enzymatic hydrolysis-derived glucose as carbon source, and single bacterial strains, that require the sterilization of the fermentation plant [2]. To compete with petrochemical plastics, it is necessary to find alternative solutions to reduce these costs such as, for example, the use of residual lignocellulosic biomass to obtain fermentable sugars and mixed microbial cultures (MMCs)[3]. For this purpose, a residual biomass collected at the end of the corn harvest was subjected to a microwave-assisted acid hydrolysis reaction (*flash*) in a multimode reactor to obtain monosaccharides at the end of the process downstream, which involves the use of ion exchange resins for the neutralization of crude reaction and the removal of undesired salts that would alter the saline composition of the fermentation broth. In particular, the conditions of the hydrolysis reaction were optimized, reducing the acid concentration to limit the volume of ion exchange resins used for neutralization and desalination. The so-obtained C5 and C6 monosaccharides were used as a carbon source for fermentation tests carried out in flasks (200 mL) with mixed microbial culture (MMC) enriched from a diary activated sludge plant in a synthetic medium containing acetic acid (20 g/L) as carbon source. The tests conducted in flask with monosaccharides as carbon source allowed to carry out a scale-up in a stirred tank bioreactor (3 L) obtaining similar results in terms of PHA yield. The extraction of PHAs from cellular biomass was carried out both with conventional protocol (sodium hypochlorite and chloroform), and with unconventional methods that involve the use of ultrasound mechanical effect for cell walls rupture. FT-IR and NMR analyses carried out on the extracted material suggest that it could be the PHBV copolymer. In conclusion, the possibility to produce PHA from residual biomass and MMCs derived from dairy activated sludge plant with the help of unconventional technologies, has been demonstrated.

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Agro-food by-products as sustainable resource: analytical approaches for bioactive compounds characterization

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The increase of global economy requires a sustainable management of agro-industrial wastes and by-products. This "living matter" represents a renewable storage of natural compounds (antioxidants, carbohydrates, enzymes) with relevant biotechnological and nutritional value. Their use is potentially applicable to different fields, as it can constitute a supplement for food products, with the effect to achieve shelf-life improvement[1]. Moreover, according to the "Zero Waste" principles, the residual matrices can be also employed for bio-composite materials production. New European regulations, promoting a limited utilization of single-use plastics, are moving towards the ecological transition in several economy fields. Unfortunately, common biopolymers show limited physico-chemical features, such as low barrier properties, and reduced stability. Biobased composite materials could overcome these limits, as agro-industrial by-products can be used as suitable fillers for mechanical properties improvement[2].

In this work, green methods were developed to extract valuable substances from by-products derived by artichokes and onions, and several analytical techniques, such as HPAEC-PAD, HPLC-SEC, GC-MS and FTIR-ATR, have been exploited for bioactive compounds evaluation and characterisation. The extracts were both proposed as food ingredients, developing new functional food with improved oxidative stability, and as additional constituent in new kind of active packaging formulation (edible film, spray, composite materials). Some of the novel materials developed also shown the possibility to be 3-dimensional printed by fused deposition[2].

In addition, the same analytical approaches play a key role in safety assessment of the developed products, allowing the detection and identification of possible xenobiotics, toxin, pesticides, and other emerging contaminants[3].

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A two-step enzymatic approach to cheese whey valorization: Synthesis of alkyl galactoside fatty acid esters as non-ionic biosurfactants

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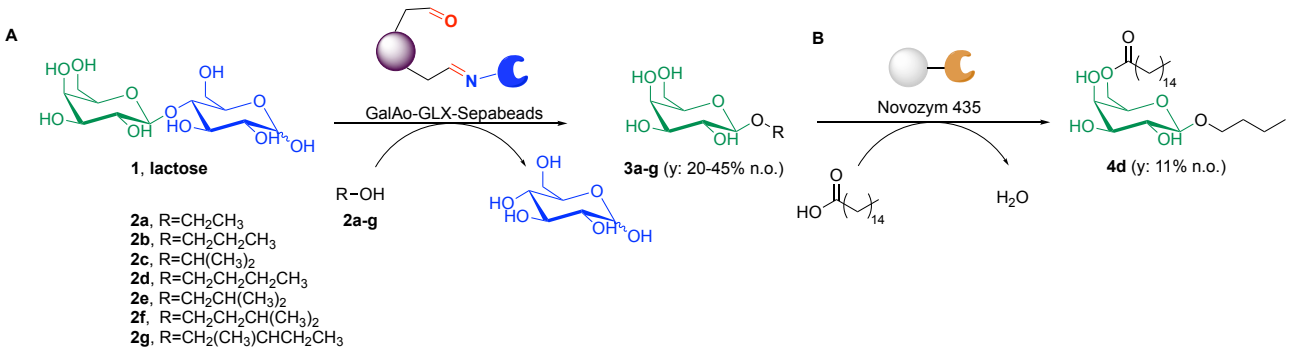
Cheese whey is the main wastestream of dairy industry. After protein recovery, the resulting whey permeate contains a pool of carbohydrates (lactose as well as its hydrolysis products, *i.e.* glucose and galactose) that can be exploited as feedstock for the synthesis of Sugar Fatty Acid Esters (SFAE), non-ionic surfactants characterized by emulsifying, stabilizing, and detergency properties.[1]
In this work,[2] an immobilization study of β-galactosidase from *Aspergillus oryzae* (GalAo) was carried out with the aim to develop a robust biocatalyst for lactose upcycling. Several types of binding chemistry, chemical activation of the support, and immobilization conditions were assayed. Glyoxyl-Sepabeads EC-EP resulted in good immobilization yields (immobilized protein=65% and immobilized activity=58%) and moderate activity recovery (20%). The immobilized GalAo was used for the synthesis of a library of alkyl-β-D-galactosides by reacting lactose with naturally occurring alcohols **2a**-**2g** through a transglycosylation reaction; compounds (**3a**-**3g**) were isolated in moderate to good yields (20-45%) (Scheme 1A). *n*-Butyl β-D-galactopyranoside (**3d**) was submitted to the esterification step with palmitic acid in a solvent-free system by using Novozym 435 as the biocatalyst,[3] affording 6-*O*-palmitoyl-1-*O*-butyl galactopyranoside (**4d**) (Scheme 1B).



Scheme 1. **A**) Transglycosylation of lactose (**1**) with natural aliphatic alcohols (**2a-g**) catalyzed by immobilized GalAo in buffer pH 4.3/alcohol/(acetone), r.t., 6 h. **B**) Esterification of **3d** with palmitic acid catalyzed by Novozym 435 in a solvent-free system, 80 °C, 8 h, molecular sieves. n.o.=not optimized.

Interfacial features and W/O emulsifying properties of **4d** together with W/O emulsion stability over time were evaluated.

Acknowledgements

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Computational Mechanistic study of Ruthenium Tetroxide Oxidation of N-Methyl-Isoxazolidine

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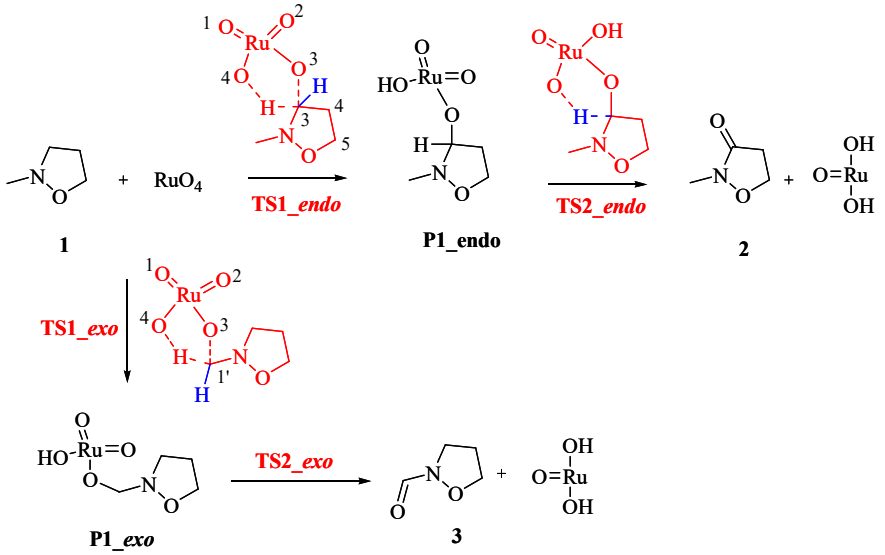
In literature, an interesting and highly selective oxidative reaction of saturated hydrocarbons, using RuO_4, was reported. In particular, the discussion was centered on the possible reaction mechanisms involved in oxyfunctionalization of cycloalkanes. In the last years, we reported the first example of a direct oxidation of the isoxazolidine nucleus to the 3-isoxazolidones using RuO_4. These compounds are valuable intermediates, useful for the synthesis of modified nucleosides that, recently, have been used as antiviral agents against the Covid-19. According to that we were interested to perform a complete computational mechanistic study of this oxidation reaction on the 2-methylisoxazolidine. The investigation was conducted taken under consideration the different sites of the substrate, where the oxidation could take place. This reaction occurs in two steps, with a double H transfer. In particular, the rate-determining one involved a [3 + 2] one-step, but asynchronous mechanism. In the first stage, when methyl propanoate is used as solvent, the formation of an ion pair that evolves to the product is located. Furthermore, the study highlighted that all the carbon atoms of the isoxazolidine system, near to the heteroatoms, can undergo the oxidation process by RuO_4, and the detected selectivity is correlated to the stability of the corresponding carbocations, leading to the N-methylisoxazolidin-3-one as preferred product.



Scheme 1

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Sustainable chemical processes: from the "green practice" of eco-friendly chemistry to a strategy for a successful Company.

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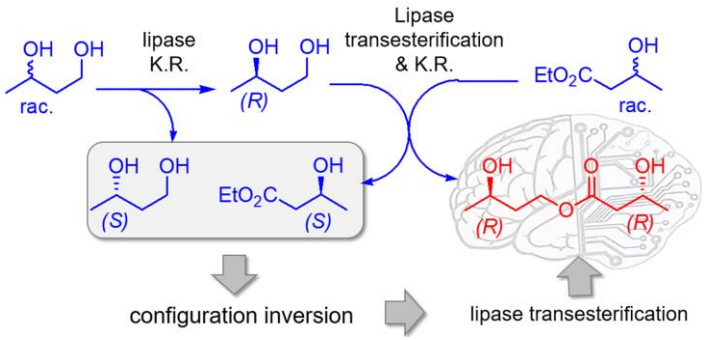
Unichem Estense srl is a Spin-off of the University of Ferrara, registered as an innovative start-up in April 2021. The company activity is the synthesis of nutraceutical products with high technological value, sustained by a high throughput research and development section, aimed to expand the portfolio of biological active molecules. The steppingstone of Unichem Estense srl is the green-chemistry technology, based on the recycling of waste material from the agricultural industry, inspired by the development an alternative strategy based on the use of cheapest starting materials, the reduction of synthetic steps (step economy) and the greater simplicity of the reactions carried out in a telescopic way. This process is in line with the circular economy based on three key principles: i) designing out waste and pollution; ii) keeping products and materials in use; and iii) regenerating natural systems. The company has already developed ketone body-like molecules to be used in the pharmaceutical and veterinary fields [1]. These molecules are produced starting from waste straw and synthetized using natural enzyme obtained from recombinant biotechnology. The production process can be traced back to three stages: i) collection and processing of straw waste; ii) synthesis of ketone bodies using biotechnological enzymes; iii) assessment of the biological activity. The market of interest is the nutraceutical one, to provide new nutritional supplements and the pharmaceutical one, based on the biological effectiveness of the different compounds, that might be employed in inflammatory and degenerative diseases. The final stakeholders are the suppliers and the global consumers, that are reassured by the quality and safety of the products.



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Green solvent extraction of biomolecules with antimicrobial activity from microalgae

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Microalgae are involved in a variety of bioactive metabolites. They manifest a great variety of biological activities. The increasing demand for drugs able to cure new diseases resistant to commonly used medicines arouses interest in unconventional new sources of bioactive natural compounds. Recent scientific investigations have revealed that microalgae compounds exhibit various beneficial biological effects such as anti-inflammatory, antimicrobial, anticancer, anti-HIV, antihypertensive, antidiabetic, and several more medicinal effects. Thus, it is of growing importance to shed light on the pharmaceutical activities of bioactive components from microalgae and to maximize their recovery from microalgal biomass. Concerning this latter point, in general, the factors that influence the extraction yield of the biological compounds from microalgae depend on the extraction method, the pretreatment of biomass, and the nature solvent used, which is the most critical factor. Organic solvents are commonly used methanol, ethanol, acetone, and ethylene glycol and their aqueous solutions. However, due to the varied physical and chemical natures of the components present in microalgae biomass, different solvents are required to optimally exploit the potentialities of the compounds produced by given microalgae. Furthermore, sustainable processes are desirable, and green solvents need to be used to replace conventional ones (Alfieri et al., 2022).

The focus of the present study was to optimize the extractions with green organic solvents. For the extractions, the freeze-dried biomass of a strain of *Chlamydomonas* was used, and in each case after adding the various solvents, the biomass was sonicated to promote cell rupture, favoring extraction. The green solvents used for extraction were methanol, dimethyl carbonate, ethyl methyl ketone, and cyclopentyl methyl ether (Alfieri et al., 2022). Furthermore, green organic solvent extractions were compared with an extraction technique using a hydroalcoholic solution and subsequent extractions with chloroform, hexane, and ethyl acetate (Yu et al., 2019). The best extraction yields were obtained with methanol and cyclopentyl methyl ether (21% and 19% weight of extract / initial dry biomass weight, respectively).

After removing all the solvent from the extract, the latter was solubilized in an aqueous solution containing DMSO and TWEEN to improve the diffusion of the extracted molecules on the agar plate. Subsequently, the extracts were tested against the model microorganism *Bacillus megaterium* using the agar well diffusion method. All the extracts obtained with green solvents showed antimicrobial activity. In particular, the best results were obtained with Ethyl methyl ketone and cyclopentyl methyl ether, which showed zones of inhibition of 2.1 cm and 2.2 cm respectively.

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Green valorisation of defatted waste of *Cynara cardunculus* L. to single cell oil and high-quality lignin

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In recent years a growing interest has been directed to 3rd-generation biomass, such as the infesting plant species *Cynara cardunculus* (cardoon). It is a very common variety in the center of Italy and in the Mediterranean region. Cardoon offers a wide spectrum of potential applications, being a rich source of fibers, oil and bioactive compounds. The cultivation of this perennial herbaceous biomass shows significant advantages, such as good adaptability to climate change and growth on marginal or uncultivated lands with modest inputs, including moderate irrigation and minimal need of nutrients. The seeds of the flower are exploited for oil production for food and bio-diesel supply chains. The nonedible lignocellulosic residues can undergo pretreatments that favour further exploitation of this biomass [1,2]. In this study, the steam exploded defatted cardoon was used as feedstock for the production of sugars-rich hydrolysates by enzymatic hydrolysis. Two different commercial enzymatic mixture Cellic® CTec2 and Cellic® CTec3 were tested at different dosages (15, 30, 45 FPU/g glucan) and in the presence of different biomass loadings (2, 5, 10 wt%). The hydrolysates obtained under optimised reaction conditions (Cellic® CTec3, 30 FPU/g glucan, 2 or 5 wt% biomass loading, 72 h) were then fermented to new generation oil by the two oleaginous yeasts *Lipomyces starkeyi* DSM 70296 and *Cryptococcus curvatus* DSM 70022. The lipid contents of 45 and 60 wt% and the lipids yields of 20 and 24 wt% were reached, respectively. The single cell oils profile was similar to that of food oils usually employed for the production of biodiesel. Finally, in order to valorise all the fractions of cardoon, a green extraction protocol was optimised in order to recover high quality organosolv lignin from exploded cardoon suitable for material applications such as dielectric for organic thin film devices and bio-based component for functional coatings (Fig. 1). Different green solvents were tested, such as EtOH, EtOH:NH_3 1:1, EtOH:H_2O 1:1, MeTHF, MeTHF:EtOH:NH_3 1.6:0.2:0.2, in an orbital shaker at 55 °C, 90 min, 50 g/L biomass loading, agitation speed 250 rpm. The lignin yield extracted by EtOH, EtOH:NH_3 and MeTHF was around 10 wt% respect to the exploded cardoon and around 30% respect to the lignin content.

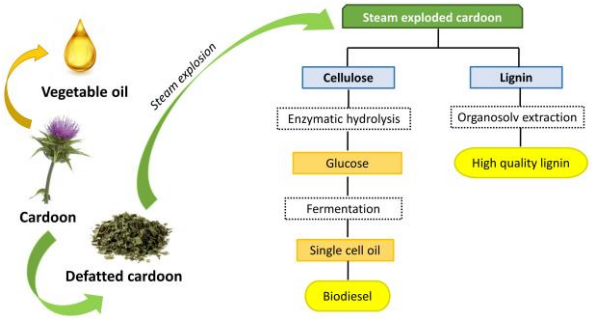

Figure 1 – Schematic representation of the implemented biorefinery scheme.

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Orthogonal nanoarchitectonics approach to engineer M13 bacteriophages as theranostic targeted platforms

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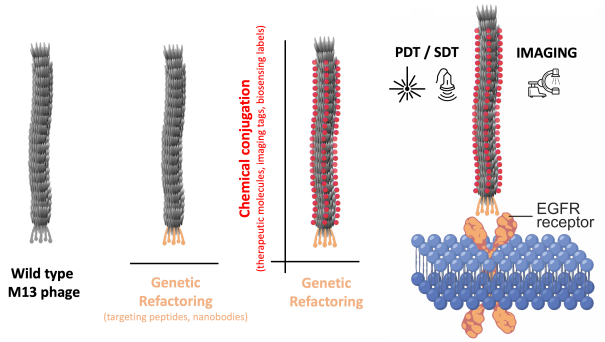
Bacteriophages, or simply phages, are ubiquitous viruses that infect bacteria but are inactive against eukaryotic cells. Phages such as the M13 phage have received growing attention as a well-defined protein-based platform for the assembly of nanostructured functional molecules and materials. They are biocompatible, very uniform in size and morphology, and relatively stable at high temperature, in a wide range of pH, and in the presence of nucleases and proteases. M13 can be genetically refactored to display on the phage tip targeting proteins/peptides. Using an orthogonal approach to the genetic display, the engineered phages can be decorated, conjugating hundreds of molecules on the viral capsid (Scheme 1).



Scheme 1

Here we show the use of engineered M13 phages as targeted vectors for efficient photodynamic/ sonodynamic killing of cancer cells. M13 was genetically refactored ($M13_{EGFR}$) to display on its tip a peptide or a nanobody able to bind the epidermal growth factor receptor (EGFR).[1,2] Hundreds of photo/sono sensitizers were conjugated on the capsid surface ($M13_{EGFR}$-PS).[1,2] The efficient retargeting of $M13_{EGFR}$ to cancer cells overexpressing EGFR was proved through flow cytometry and confocal microscopy experiments.[1,2] The killing activity of cancer cells is observed at picomolar concentrations of the phage vector in both PDT/SDT modality.[1,2]

The developed orthogonal (genetic/chemical) strategy for engineering M13 bacteriophages demonstrates that (i) the phage tropism of M13 may be easily varied to target different receptors; (ii) theranostic platforms may be developed conjugating both therapeutic and imaging tags on the phage capsid.

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An argininocalix[4]arene for the effective delivery of miRNAs

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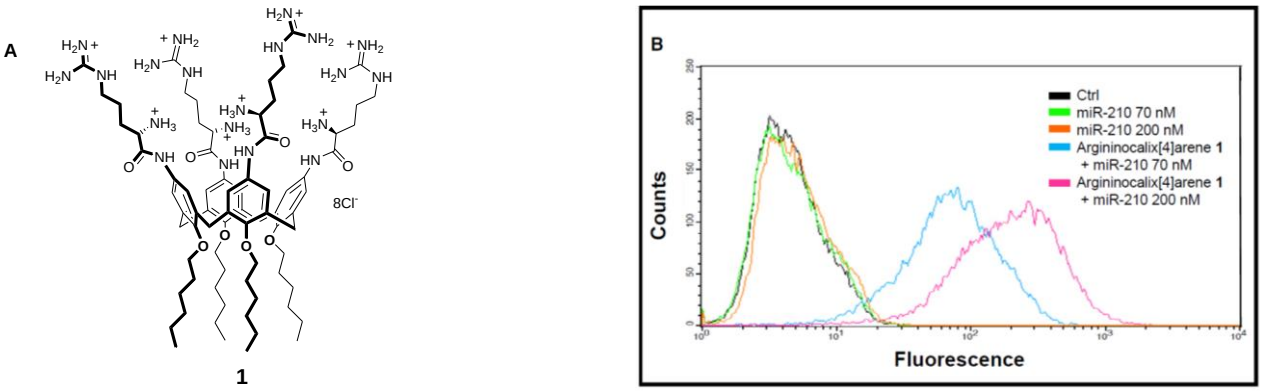
MicroRNAs (miRNAs) are short non-coding RNA molecules acting as gene regulators by repressing translation or by inducing degradation of the target RNA transcripts.[1] Altered expression of miRNAs may be involved in the pathogenesis of many severe human diseases,[2] opening new avenues in the field of therapeutic strategies, i.e., miRNA targeting or miRNA mimicking.[3] In this context, the efficient and non-toxic delivery of premiRNA and antimiRNA molecules is of great interest. In the last years we designed a series of non-viral vectors based on positively charged calixarenes that were demonstrated to be extremely efficient transfection agents for the delivery into the cells of plasmid DNA[4] and Peptide Nucleic Acids,[5] interesting mimics of the natural nucleic acids. The tetraarginino calix[4]arene **1**, in particular, resulted the lead compounds in this biological activity. Due to the potential therapeutic relevance of miRNAs, we explored the ability of **1** to transport these derivatives into cells and will present our encouraging results. We indeed verified[6] that (1) the toxicity of argininocalix[4]arene **1** is low, and it can be proposed for long-term treatment of target cells, being that this feature is a pre-requisite for the development of therapeutic protocols; (2) the delivery of miRNA, premiRNA and antimiRNA molecules is efficient, being higher when compared with reference gold standards available; and (3) the biological activity of the delivered short nucleic acids is maintained.



A) The argininocalix[4]arene **1** used as vector and B) the internalization of miR-210 in absence and in presence of **1**, as determined by FACS analysis.

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Optimizing Novel Cholesterol Nanomedicines for Brain Delivery

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Treating Central Nervous System (CNS) disorders, such as neurodegenerative diseases, represent one of the most hampering challenges in the medical field. Despite the fact that many drugs have shown potential curative effect against these pathologies, there are still many obstacles to be overcome that hinder the entrance of these active compounds into the brain, above all the Blood-Brain Barrier (BBB). In this view, nanomedicines (NMeds) could be a powerful tool to deliver therapeutics adding the possibility for surface-engineering with properly selected ligands to enable BBB crossing and penetration into the brain. In this work, novel lipid-based NMeds composed of cholesterol (Chol) were formulated according to nanoprecipitation procedure and fully characterized in terms of physico-chemical, technological, and morphological properties. These NMeds showed a particle size < 300 nm and a polydispersity index < 0.3, showing suitability for systemic injection. Chol NMeds were then formulated with the incorporation of three novel potential CNS-targeting ligands (ligand 1, ligand 2, and ligand 3) and, for each targeted NMed, an optimization was performed by varying the main formulative parameters, such as organic solvent, amount of ligand used in formulation, amount of surfactant in the aqueous phase, and purification method. Chol-ligand 1, Chol-ligand 2 and Chol-ligand 3 NMeds were physico-chemically, technologically and morphologically characterized, showing a particle size < 300 nm and a narrow polydispersity (PdI < 0.3). Optimizations are also being performed to determine the amount of each ligand that remains in the final formulation using HPLC quantification. This work paves the way to future drug loading studies: optimized CNS-targeted Chol NMeds will be loaded with three drugs with different physico-chemical properties, and tested *in vivo* to confirm the efficacious brain penetration, leading to novel nanocarriers for improved drug delivery to the CNS.

MICROFLUIDIC TECHNOLOGY OPTIMIZATION FOR THE FORMULATION OF PEPTIDE-LOADED LIPOSOMES

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Microfluidics is an emerging nanotechnological field which offers the possibility to create uniform, monodispersed, and easily scalable nanoparticles using chips with different architectures, for example circular or zig zag. In particular, this technology has recently demonstrated great potential in the production of liposomes.

In this study, three different types of liposomes were optimized utilizing a microfluidic device for the encapsulation and delivery of a neuroprotective peptide: 1) neutral liposome of dipalmitoylphosphatidylcholine (DPPC) and cholesterol; 2) cationic liposomes of DPPC, cholesterol and 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP); and 3) anionic liposomes consisting of DPPC, cholesterol and 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylglycerol (POPG).

The liposome formation was optimized by varying factors such as the molar ratios between the lipids, and flow rate ratios (FRR, 1:1, 1:0.5, 1:0.3) in order to tune the characteristics of the liposomes. The final volume of the formulation, the total flow rate, the maximum concentration of liposomes, and the solvent (methanol) were kept constant. The liposomes were characterized using photon correlation spectroscopy to detect size, monodispersity (PDI), and the surface charge (Zeta-potential). Morphology of the liposomes was also determined using Atomic Force and Electron Microscopy and the encapsulation efficiency of the peptide was evaluated via HPLC analysis. Furthermore, liposome stability to different storage conditions (freezing -20°C, or +4°C) was investigated.

These optimizations will help us create stable and scalable liposomes that can deliver huge amounts of this peptide. In the future, the most promising optimized formulations will be tested in mice models of retinal degeneration, to assess the efficacy and activity of the peptide-loaded liposomes.

Proteins as Trojan Horse for biomedical applications of fullerenes

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Proteins behave as supramolecular hosts able to recognize molecular nanoparticles directly in water, resembling a typical biomolecular complexation (ligand-protein complex formation).

Fullerenes are candidates for theranostic applications because of their high photodynamic activity and intrinsic multimodal imaging contrast. However, fullerenes suffer from low solubility and a tendency to aggregate in aqueous media, poor biocompatibility, and cell toxicity.

Noncovalent bioconjugation of fullerenes with proteins is an emerging approach for their dispersion in aqueous media.[1] Contrary to covalent functionalization, the supramolecular approach preserves their molecular structure and physicochemical properties. The unique photophysical and photochemical performances of fullerenes are then fully accessible for applications in different fields, from materials science to nanomedicine.

The hybridization of fullerenes with proteins allows the production of innovative multifunctional theranostic platforms where the role of proteins is akin to that of "Trojan Horses" since they can i) hide the fullerene from the biological *milieu*, ii) control their cellular uptake, and iii) govern their biological fate.[2]

The exploitation of C_{70} fullerene as a phototheranostic agent has been demonstrated by the C_{70}@lysozyme supramolecular complex. The excellent imaging contrast of C_{70}@lysozyme in optoacoustic microscopy was exploited to monitor its uptake in HeLa cells and its lysosomal localization. The photodynamic activity of C_{70}@lysozyme caused cell death through reactive oxygen species production upon exposure to low-intensity white light irradiation.[3]

A similar approach can be extended to a wide range of insoluble nanomaterials, spanning from other carbon nanoparticles (i.e., carbon nanotubes), to different molecular nanoparticles (i.e., gold nanoclusters, carboranes), allowing their manipulation in aqueous media for exploitation different biomedical fields.

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Whole-cell biotransformation of 3-chloropropiophenone by microalgae *Chlorella emersonii* entrapped in hydrogel

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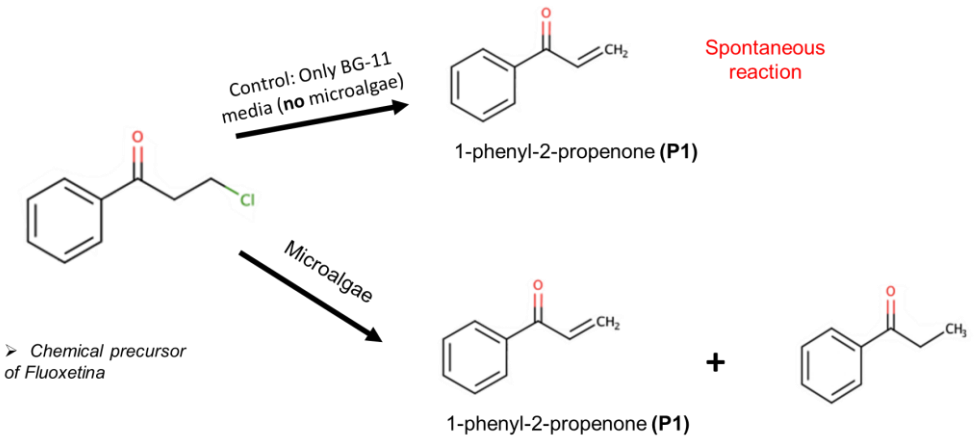
Dehalogenation of halogenated organic substrate (3-Chloropropiophenone, 3-CPP) is conducted using whole cells microalgae *Chlorella emersonii* (211.8b) as biocatalyst. *C. emersonii* cells were immobilized by entrapment in Aquasorb (an anionic polyacrylate received from SNF Italia able to absorb water up to 400 times the dry weight) and used to catalyse the biotransformation of the toxic 3-chloropropiophenone. The reaction produced 91% or 93.5% 1-phenyl-2-propenone (from a non-catalyzed reaction) and 9% or 6.5% 1-phenyl-1-propanone thanks to the biocatalyzed reaction with entrapped or free (non-immobilized) whole microalgae cells, respectively. The viability of *C. emersonii* in the presence of 3-CPP was monitored by analysing the fluorescence emitted by the chlorophyll of microalgae. The decrease of fluorescence of the culture of microalgae in the presence of 3-CPP (and the derivatised products) indicates the destruction of chlorophyll and cell death (90% after 52 h with 5 mM of 3-CPP) after which the ratio between the two products (0.36 ± 0.02) remained unaltered, suggesting the inhibition of the biocatalysed reaction. Instead, in the same conditions, the hydrogel entrapped cells show viability in terms of chlorophyll fluorescence even after 52 h. Thus, it can be suggested that the higher biotransformation of 3-CPP to 1-phenyl-1-propanone with the entrapped cells depends on the higher cell viability. In conclusion, the results did not confirm that the biotransformation to 1-phenyl-1-propanone is due to dehalogenase or hydrogenase activity. Instead, it appears correlated to the antioxidant concentration of the microalgae culture media.[1] The study indicates that the immobilization of microalgae in Aquasorb (and likely also in other types of hydrogels) can be a procedure for improving microalgal applications in biotransformation or bioremediation processes.



Scheme 1

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Catalytic lipophilization of natural antioxidants

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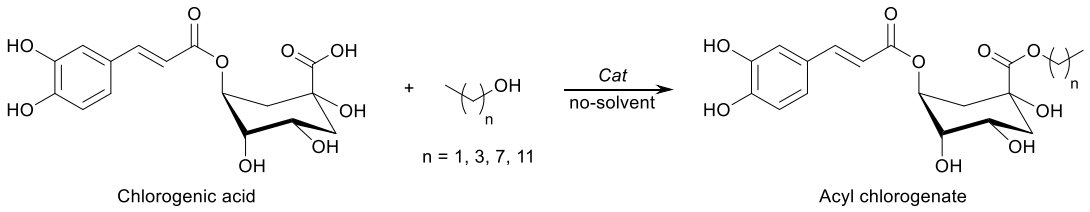
The valorization of agro-food industry residues is a pillar of circular economy. In the framework of project CirCo aiming at enhancing silverskin, a residue of the coffee roasting industry with almost no application, several products are under development. Among these, chlorogenic acid (CGA),[1] a natural polyphenol widespread in nature (plants, vegetables, fruits), displays several pharmacological and biological activities such as antioxidative, antibacterial, antihypertensive, antitumor, anti-inflammatory.[2] However, its highly hydrophilic structure strongly limits its bioavailability and hinders the use into oil-based products such as cosmetic and nutraceutical preparations.

To overcome this drawback, CGA can be lipophilized through esterification with a fatty alcohol.[3] The use of heterogeneous Brønsted solid acid catalysts, such as the sulphonic resins Amberlite® IR120 and Amberlyst® 15, resulted greatly effective in the direct acylation of CGA with several fatty alcohols (2 to 18 carbon atom) in a sustainable, solvent free, one-pot reaction (Scheme 1).



Scheme 1

Ethyl, butyl, octyl- and lauryl- chlorogenates were isolated in high yields (up to 93%) and fully characterized by NMR and LC-MS analyses. The antioxidant activity of these products has been studied and compared to unsubstituted CGA (Figure 1).

Pilot studies have shown that the ethyl ester of CGA is able to protect human fibroblast cells from the effects of aging and to preserve their activity following UV-induced oxidative stress.

Preliminary reactions highlighted the applicability of this method also to the esterification of mono- and oligo-saccharides.

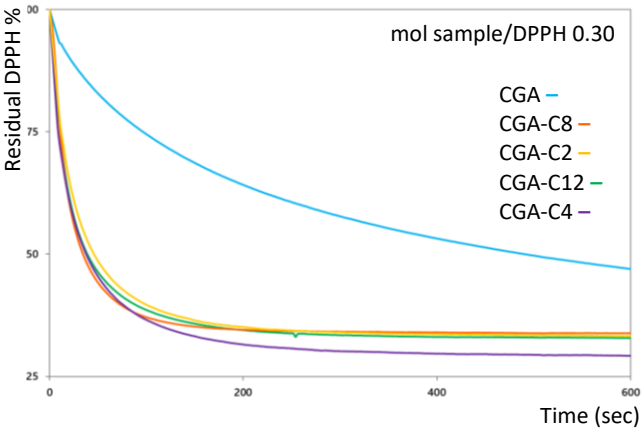


Figure 1

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Biocatalytic process for the production of enantiomerically enriched vicinal diols: from *in vitro* to *in vivo* system

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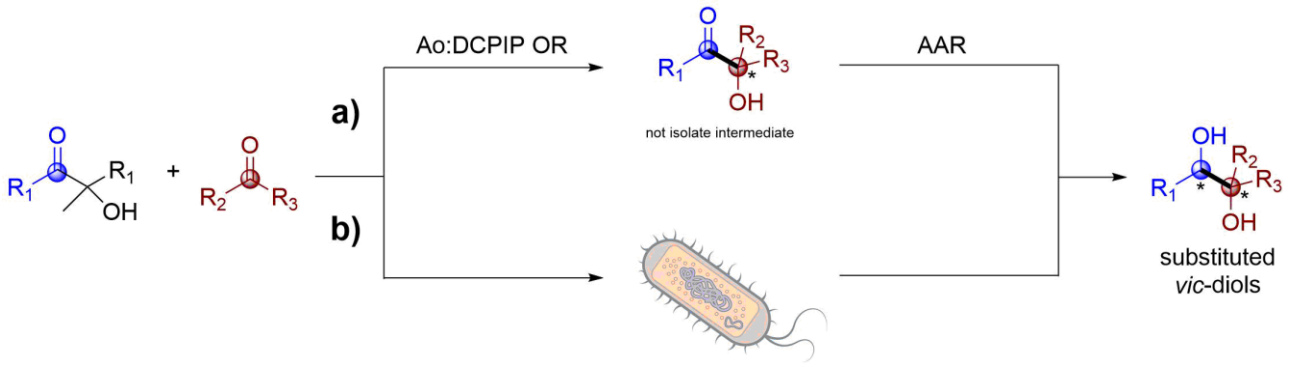
In recent years the group of Organic Chemistry of University of Ferrara has highlighted the synthetic relevance of the thiamine diphosphate dependent lyase acetylacetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) and of the NADH dependent reductase acetylacetoin reductase (AAR) both isolated from *Bacillus licheniformis* DSM13 and then cloned into a bacterial overexpressing cell.

Recently an enzymatic one-pot two-step procedure for the preparation of enantiomerically enriched *vic*-diols based on the combined used of the above enzymes has been proposed[1]. Thanks to this fully enzymatic approach a broad library of variously substituted enantiopure *vic*-diols has been obtained. Some of the synthetized *vic*-diols are known for their biological activity. Among them the ethyl ester of 2,3-dimethylglyceric acid caught our attention because of the widespread presence of its acyl moiety in many natural compound structures. The absence of information on the absolute stereochemistry of this compounds moved us to design and implement a chemo-enzymatic route for the synthesis of all the four stereoisomers of the 2,3-dimethylglyceric acid ethyl ester[2].

Today our efforts are focused on the switching of the biocatalytic synthetic pathway from an *in vitro*, to an *in vivo* methodology. During the oral contribution will be illustrated our advances in the construction of a whole cell biocatalyst holding the complete enzymatic machinery (Ao:DCPIP OR, AAR and a NADH recycling enzymes).



Scheme 1. A) Isolated enzymes route B) Whole cellular biocatalyst route

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From dairy waste to hexose-derived building blocks

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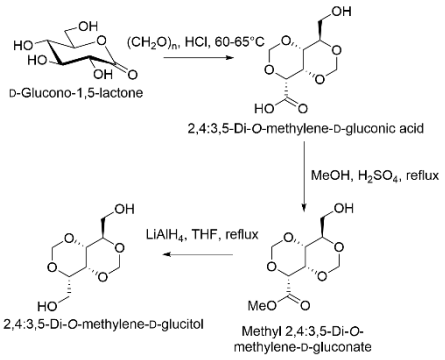
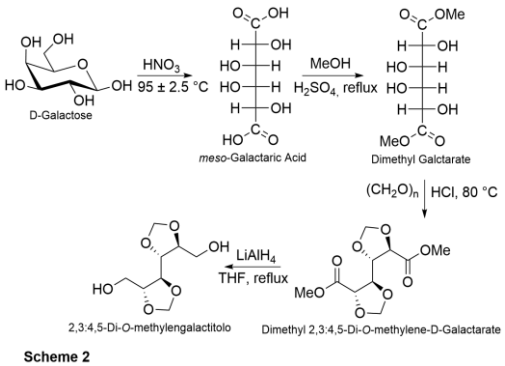
The process of cheese-making in Italian dairy industry leads to the stockpile of an abundant by-product, cheese-whey (CW). An ultrafiltration treatment can separate the protein fraction of whey (CW retentate), used in food and feed industries, from the sugar fraction (CW permeate), that is more difficult to exploit. Our project focuses on the valorisation of the main CW permeate component (*i.e.*, lactose) to obtain a library of building-blocks for chemicals synthesis. Firstly, lactose must be divided into its constituent monomers (*i.e.*, D-glucose and D-galactose). The hydrolysis can be efficiently carried out with a cold-active β-galactosidase (β-Gal)[1], whose activity at low temperatures makes it suitable for low-energy processes. In a circular economy perspective, the β-Gal was recombinantly produced in *E. coli* using CW permeate to induce its expression. The derived hexoses are then separated[2]. To produce added-value chemicals (*i.e.*, copolymers, plasticisers, industrial additives etc.) building blocks must have thermal stability and good mechanical properties, thus chemical modification of the functional groups of the hexoses is required[3]. The synthetic strategy for the modification of D-glucose is the following (**Scheme 1**): it is first converted to D-glucono-1,5-lactone by enzymatic oxidation[4]; than the secondary hydroxyl groups are protected with paraformaldehyde in a reaction catalysed by HCl 37%, yielding a rigid bicyclic aldonic acid[5]; subsequent esterification reaction with methanol, catalysed by concentrated H_2SO_4, yields a protected aldonic acid ester[6], and finally a reduction with commercial catalysts such as $LiAlH_4$ yields a protected alditol[7]. The synthetic strategy for D-galactose (**Scheme 2**) starts with an oxidation with HNO_3 to obtain *meso*-galactaric acid (mucic acid)[8]. The following step is the protection of the secondary hydroxyl groups to improve thermal stability, however the reaction with paraformaldehyde was unsuccessful because galactaric acid has low solubility in water[9]. A possible way to improve this parameter is the esterification of galactaric acid with methanol catalysed by concentrated H_2SO_4, to obtain dimethyl galactarate[10]. An alternative is to improve solubility by raising temperature and use a carbonyl compound for the protection that does not evaporate using a condenser, like acetone. One more possible step is to reduce protected dimethyl galactarate with $LiAlH_4$ to produce protected alditols[11].



Scheme 1



Scheme 2

Acknowledgements. This work has been supported by Fondazione Cariplo, grant n° 2020-0838.

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Microalgae potential in the capture of carbon dioxide emission

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Although industrialization has improved our daily lives, the impact on the environment in terms of carbon dioxide emissions has worsened. For example, the contribution of fossil fuel power plants appears to be about 40% of the total global emissions of carbon dioxide, a greenhouse gas with a significant negative impact on global warming, to which must be added the combustion of fossil fuels for transport. In heavy industries, carbon dioxide emissions are a by-product of chemical reactions that do not involve combustion, but carbon dioxide emissions indirectly released by the production of electricity must also be considered.

The use of versatile and environmentally friendly photosynthetic organisms, such as microalgae, represents a promising approach in the development of carbon dioxide abatement systems.

In the present study four microalgal strains (*Chlorella vulgaris Beij.* ACUF 863, *Chlamydomonas pitschmannii Ettl* ACUF 292, Scenedesmus sp. ACUF 145, Scenedesmus sp. ACUF 329) from the Algal Collection University Federico II were cultivated at different carbon dioxide concentrations.

The strains were grown in a Multi-Cultivator MC 1000-OD, with BBM+V as growth medium. The different growth conditions tested by varying the light wavelength and the carbon dioxide concentration have shown that Scenedesmus sp. ACUF 329 was particularly promising for the carbon dioxide capture.

In fact, microalgae Scenedesmus sp. ACUF 329 in the presence of 5% carbon dioxide concentration exposed to warm white at 2700K or red light at 660nm produces 181 $mgL^{-1}d^{-1}$ and 279 $mgL^{-1}d^{-1}$ of dry biomass respectively, compared to what happens in air, where it is produced around 26 $mgL^{-1}d^{-1}$ both in white and red light. The most interesting data is the one related to the consumption of carbon dioxide by the microalgae during growth, which turns out to be 332 $mgL^{-1}d^{-1}$ and 404 $mgL^{-1}d^{-1}$ in the presence of 5% of carbon dioxide concentration, in white and red light, respectively, as against around 45 $mgL^{-1}d^{-1}$ when the carbon dioxide is that present in air, both in white and red light.

It is therefore clear how the microalgae Scenedesmus sp. ACUF 329 have a great potential in the biofixation of carbon dioxide in microalgal biomass, which will be exploited in terms of productivity linked to the presence of the lipid component.

Evaluation of the effects of fermentation on carbohydrate profile in low-FODMAP baking products

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The irritable bowel syndrome (IBS) is one of the most popular gastrointestinal diseases affecting the 10 – 20 % of the European and American populations[1]. Since a pharmacological therapy for patients treatment is not available, the only approach is to follow a low-FODMAP diet. FODMAPs – Fermentable Oligo-, Di-, Mono-saccharides and Polyols – are carbohydrates which are fermented from colon bacteria[2]. They are generally known to have prebiotic activities providing an excellent substrate for the elective nourishment of microbial species. However, for individuals with functional gastrointestinal disorders, such as IBS, their ingestion can trigger symptoms. The development of low-FODMAP food, such as bakery products, is encouraged.

This work focused on the characterization of carbohydrates in different bakery products provided by BRU.PI srl, a start-up that proposes new formulations to achieve low-FODMAP products. High Performance Anion Exchange Chromatography with Pulsed Amperometric Detector (HPAEC-PAD) is a useful analytical tool to study the carbohydrates patterns[3]. Indeed, this technique is selective for sugar separation and identification, and can be focused on oligosaccharides or simple sugars, by using appropriate chromatographic columns. In particular, in this work the use of different columns able to focus on the presence of oligosaccharides, or to perform quantitative analysis of mono- and di-saccharides was exploited. Samples of doughs and cooked products were evaluated in order to assess their composition and to select the best conditions to produce low-FODMAP foods. The quantitative analysis allowed to establish whether the amount of FODMAP occurring in final products was below the *cut-off* limit of 3 g per day[4].

The obtained results evidenced that the carbohydrate pattern, including FODMAP content, depends on different technological parameters, as type of yeast and mix of bacteria used, time of fermentation, temperature, pH, and cooking modalities. Therefore, the selection of proper conditions can permit to tailor the composition of the final product in order to obtain low-FODMAP foods.

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Peptide-based hydrogels for biotechnological applications: tunable and multivalent matrices for tissue engineering

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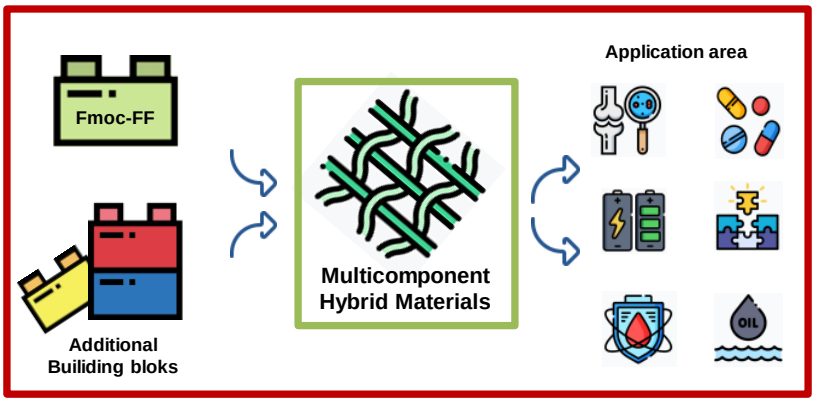
In the material science field, many peptide-based building blocks have attracted interest as suitable chemical entities for the developments of nanostructured architectures, including nanosferes or nanofibers.[1,2] As consequence of a multiscale aggregation phenomenon, some of them were found able to form tridimentional self-supporting material with a no-Newtonian flow behaviour, namely hydrogels (HGs). According to their structural and functional properties (*e.g.,* rigidity, elasticity, injectability), HGs are applied in different areas, including industry, biomedicine, and biotechnology (Scheme 1). Starting from some structural and model studies, we synthetized a series of novel low molecular weight peptide-hydrogelators based on the polymer/peptide PEG8-F6, an hexaphenylalanine with a PEG moiety at its N-terminus.[3] All the derivatives contain natural (Tyr, Trp, Phe) or unnatural (Nal, Dopa) aromatic residues.[4,5] Material structural characterization pointed out a direct correlation between the sequence and the functional properties. To improve the rigidity, we also applied a co-assembly strategy with Fmoc-FF hydrogelator.[6] Inserting non-coded amino acids, polymers chain and modifying the gelation environment, we were able to obtain gel materials with the desired mechanical response for tissue engineering applications, stable formulation of hydrogels and hydrogel particles for bioimaging, responsive oxidative matrices and UV-Vis cross-linkable hybrid materials.



Scheme 1. Peptides can be used as molecular Lego to produce hydrogels, multivalent tools applied to different technological areas.

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Modification of polysaccharides for a new generation of renewable plastics

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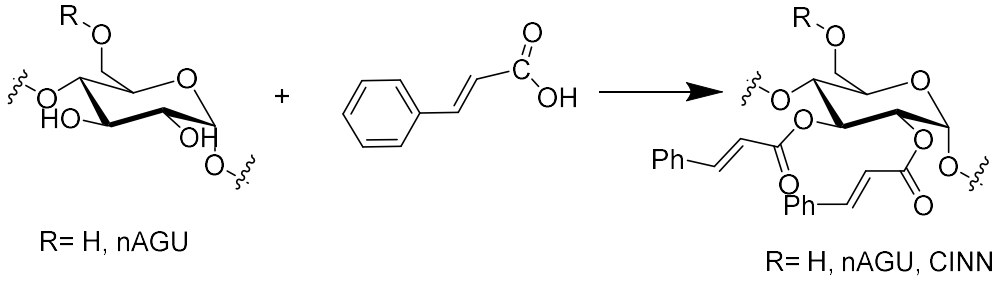
Polysaccharides natural polymers are very abundant in nature and available in most part of heart and seas. Among polysaccharides starch is capturing attention because of its large availability in nature. Starch is an example of renewable resource suitable as raw material for plastic consumer market. Compared to plastics derived by fossil resources it has the advantages of biodegradability, biocompatibility and possible conversion to energy source such as biogas. Nowadays modified starch has not yet mechanical robustness and chemical inertness to be competitive with plastics present on the market, however the research is intense and with interesting sparks.

Some research activities concern the modification of starch with natural molecules. Cinnamic acid, in particular, is characterized by anti-inflammatory properties. The studies are devoted to the synthesis of an augmented polymer by merging the biocompatibility with the therapeutic properties of the functional group. Present work study different strategies for the modification of starch (Scheme 1) in order to introduce the cinnamyl group. Moreover this group allows the photoinduced cross-linking of starch improving mechanical properties. Modification of starch is accomplished by different chemistries in order to optimize the degree of substitution (DS), defined as the average number of functional groups introduced in the anhydro glucose unit (AGU).

DS is the key parameter to quantify the modification of the polymer and ^{1}H-NMR is the chosen for this determination, preferred to titration that suffers from lack of accuracy and reproducibility.[1] In the ^{1}H-NMR analysis the dissolution of the sample and the presence of hydroxyl groups make hard the accurate DS determination.[2]



Scheme 1

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Characterization of poly (butylene-adipate-terephthalate) – calcium-phosphate glass composites: viscoelastic properties and degradation

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The aim of this work was the characterization of the viscoelastic properties and the study of the degradation of composites made of poly (butylene-adipate-terephthalate) (PBAT) filled with different amounts of calcium-phosphate glass (CPG) microparticles. The composites were previously realized with CPG contents equal to 0 wt% (PBAT), 4 wt% (PBAT+4% CPG), 10 wt% (PBAT+10% CPG) 20 wt% (PBAT+20% CPG), and 40 wt% (PBAT+40% CPG). Injection-molded standard 1BA specimens and compression-molded films were utilized for dynamic-mechanical analysis (DMA) and creep characterizations, and accelerated weathering (AW) and disintegration tests, respectively. AW consisted of combined exposure to UV radiation, high temperature, and humidity, to mimic environmental conditions, while the disintegration test was performed in a laboratory-scale composting condition.

DMA allowed assessing an increase in the stiffness of the composites with increasing filler content, observable through a homogeneous increase in the Storage modulus (E') values. Besides, thermal stability was maintained, as the glass transition temperature (T_g), corresponding to the peak of the Tanδ curves, didn't exhibit a marked trend. The increased stiffness was confirmed by a decrease in the creep compliance (J), recorded under constant stress, as the CPG content increased, in the full tested temperature range.

Samples subjected to AW showed an increased degradability as a function of the filler content, with a marked tendency to yellowness and increased color variation rate, measured through CIE L^*a^*b colorimetry. Scanning Electron Microscopy (SEM) and Electron Dispersion Spectroscopy (EDS) enabled to highlight any surface modifications of the samples in terms of appearance and composition, respectively. Fourier-Transform Infrared Analysis (FT-IR) revealed an increased modification of the spectrum as a function of both time of exposure and CPG concentration, mainly due to water absorption and photo-degradation.

The disintegration test, as well as the AW, revealed an increasing degradation rate, corresponding to the coefficient of the mass loss exponential fitting law. The degree of disintegration (D) reached at the end of the testing period was between 38% and 79%, corresponding to PBAT and PBAT+40% CPG respectively, with an increasing trend. In this case, a de-structurization of the spectrum could be observed, corresponding to increased degradation of the materials as the test proceeded.

Low-density polyethylene (LDPE) was tested for both degradation tests as a non-degradable polymer reference.

The realized PBAT-CPG composites present therefore modulation of all tested properties according to the filler content and can be considered sustainable alternatives for non-biodegradable thermoplastic polymers.

SYNTHESIS AND CHARACTERIZATION OF AN ANTISENSE PNA TO DOWNREGULATE THE PD-L1 PROTEIN OVEREXPRESSION IN CANCER CELLS

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Cancer immunotherapy is focused on stimulating the immune system against cancer cells by exploiting immune checkpoint mechanisms. PD-1/PD-L1 is one of the most known immune checkpoints due to its widespread deregulation in cancer tissues. PD-L1 overexpression was highly demonstrated to represent tumor status, favoring its survivor, and spreading. The down-modulation of PD-L1 expression on tumor cell membranes restores the immune system activation against cancer cells, and because of this, several anticancer therapies are based on anti-PD-L1 or anti-PD-1 monoclonal antibody administration. The lack of specificity and the high cancer therapeutic resistance related to the monoclonal antibody treatments pushed the advancement of novel immunotherapy approaches. With this aim, we proposed an antisense strategy to regulate the PD-L1 expression in tumor cells by targeting PD-L1 mRNA. A Peptide Nucleic Acid (PNA) was synthesized as Antisense Oligonucleotide (ASO) due to its enhanced thermal and enzymatic stability compared to its natural counterparts (1). The addition of a six Lysine residue tail at the PNA amino-terminus allowed its complexation with an oncolytic Adenovirus 5/3 Δ 24 elected as a suitable delivery system (2), taking advantage of the specific-tissue response mediated by the viral oncolytic skill of infecting and killing tumor cells exclusively. The antisense activity of the PNA was tested in comparison with the activity of a Scrambled PNA. The expression level of PD-L1 protein was measured by Flow Cytometry analysis after two days of treatment both in the presence and absence of the Adenovirus delivery platform. The decreased expression of PD-L1 protein on the cell surface was detected in the SK-OV3 cell line only for the cells treated with the antisense PNA delivered by the oncolytic Adenovirus, along with further confocal microscopy confirmation of the PNA cytoplasmatic delivery.

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Peptide nucleic acids – peptide conjugates as tools for targeting miRNA-mediated drug resistance in chronic myeloid leukemia

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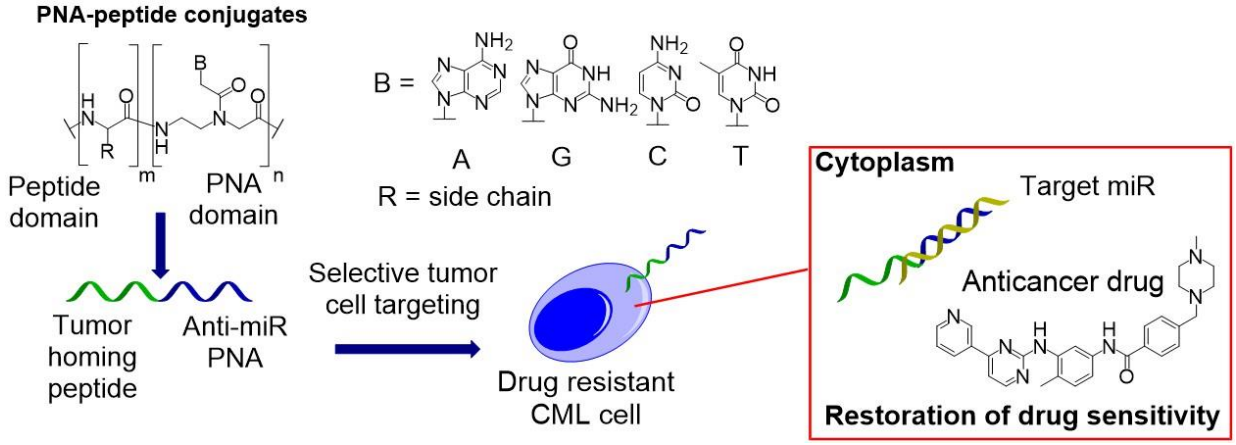
Resistance or intolerance towards chemotherapy are frequently reported in chronic myeloid leukemia (CML), and, in several cases, have been connected to the aberrant regulation of microRNAs (miRs).[1] Peptide nucleic acids (PNAs)–peptide conjugates are tools for the knockdown of oncogenic miRs, in which the PNA domain blocks the selected counterpart by complementary base pairing and the peptide domain provides an improved cell uptake.[2] Hence, the rational design of the former and the latter domain can be used to modulate the affinity for different molecular targets and cell populations, respectively. This strategy is currently studied in the frame of a project founded by "Associazione Italiana Ricerca contro il Cancro" (AIRC), aimed at tackling miR-mediated drug resistance in CML (**Scheme 1**).

Here is described the first stage of this project, consisting in the screening of different PNA and peptide sequences to generate a first array of tailored conjugates. A small library of *tumor-homing* peptides[3] was in fact obtained to verify their ability to discriminate between healthy and leukemic cell, in comparison to a non-selective *cell-penetrating* peptide (i. e. octa-arginine). The synthesis of a series of PNAs bearing anti-miR sequence is instead in progress to validate their binding partners as targets to restore drug sensitivity. Upcoming cellular experiments will point out the best sequences within the two tested libraries, that will be used as PNA and peptide domains to give the corresponding conjugates. The ability of these molecules in combining strong anti-miR activity, selective accumulation into CML cells lines, and the ability to cooperate with anticancer drugs (i. e. tyrosine kinase inhibitors) will be assessed in the following steps of this project by means of advanced cellular models (i. e. 2D and 3D static or dynamic co-cultures).



Scheme 1: schematic representation of miR-mediated drug resistance repression by means of tailored PNA-peptide conjugates into CML cells.

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Expression, purification, and characterization of Transcriptional Enhancer Associated Domain (hTEAD4), a promising target for anticancer agents

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Protein preparation for a broad technological application represents one of the highest expertise requests in the biotechnology research and application. Our work is focused on the optimization process for the preparation of a novel non-catalytic protein from the Hippo Pathway, necessary for anticancer research studies.
The Hippo Signalling pathway represents an emerging topic in tumour suppression regulation and regenerative medicine. The pathway is activated by extracellular anti-proliferative signals and is finely regulated by a cytosolic phosphorylation cascade of four main proteins with serin-threonine kinase activity, LATS, MOB, MST1/2 and YAP (YES Associated Protein). The downstream phosphorylation of YAP/TAZ paralogues proteins act as TEAD1-4 (Transcriptional Enhancer Associated Domain 4) transcriptional coactivators, while YAP Ser127 phosphorylation activates YAP/TAZ proteasomal degradation, thus preventing its migration to the nucleus for YAP-TEAD interaction, and the transcription of genes activating cell proliferation [1].

Despite being a promising pharmaceutical target, the disruption of YAP-TEAD complex is still under preliminary screenings, and further investigations are necessary to better understand how the pathway can be inhibited. Moreover, the difficulty in obtaining enough recombinant protein is a limiting factor in Hippo Pathway drug discovery and there is a need to optimize its preparation process. This requires the exploration of different purification technologies. Herein, we have developed an efficient purification protocol transforming ArcticExpress (Agilent) competent cells, able to grow at low temperatures, with pET15b plasmid encoding for 6xHis-Tagged hTEAD4 YAP binding domain (aa 217-434). The transformed cells are grown at low temperature in autoinducing enriched medium for 60h. The target protein is purified by the harvested cells in a four-step protocol consisting of two His-trap nickel-affinity chromatography and two desalting steps. The overall yield is 40mg/L of bacterial culture.

Protein is characterized with LC-MS in its acylated and non-myristoilated form. PTM's (phosphorylation) are mapped through bottom-up sequencing after trypsin hydrolyzation. Having no catalytic activity, its correct folding has been validated proving its ability to bind a YAP-mimicking peptide [2] with a FRET assay (Förster resonance energy transfer). The assay requires the conjugation of both purified hTEAD4 and YAP-mimicking peptide with fluorescence probes, fluorescein and tetramethyrhodamine respectively, on two exposed and reactive cysteines. The complex formation is confirmed by the emission of tetramethylrhodamine due to non-radiative energy transfer, allowed by the proximity of the probes, once fluorescein is excited.

Furter steps will include the development of a YAP-TEAD displacement assay to test the ability of a library of molecular disrupters to dissociate the complex.

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Evaluation of carboxylation degree on osteocalcin

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Human osteocalcin (OC), the most abundant non-collagen protein in bones, is a 49 amino acids protein synthesized mainly in the osteoblasts[1]. This polypeptide is produced as a pre-pro-peptide that undergoes several post-translational modifications before its secretion into the bone matrix[2]. Among them the vitamin-K-dependent carboxylation of three glutamate residues (Glu→Gla), at positions 17, 21 and 24, leads to a greater affinity for Ca^{2+} ions and to a conformational transition from an unstructured random coil to a folded protein[1]. Moreover, these Gla residues occupy positions complementary to the Ca^{2+} sites presented in the hydroxyapatite crystal, involved in the formation of a high-affinity mineral-protein complex in bones[3]. Therefore, due to the unstructured random coil, undercarboxylated OC does not bind to hydroxyapatite and it is leakage into the blood[4].

To better understand how a small chemical modification, like γ-carboxylation, can affect OC properties and structure, different carboxylate OC forms has been investigated. Their migration properties have been evaluated through both SDS-PAGE and native electrophoresis; while OC forms stability, structure and Ca^{2+} binding properties have been investigated by mass spectrometry, circular dichroism spectroscopy and calorimetry.
The results obtained under different analytical techniques showed that the carboxylation is stable and in certain residues promotes calcium interaction.

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Development of a sensitive biochemical tool to assess the expression levels of recombinant ectopic proteins *in vitro* engineered cellular systems

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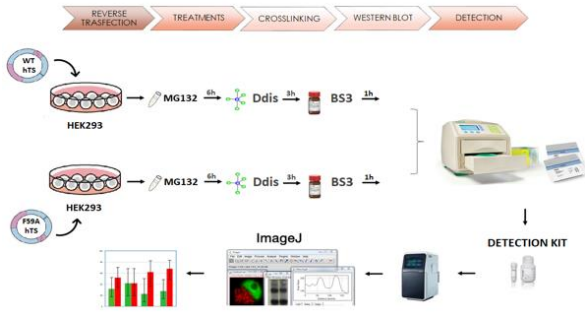
The detection of specified proteins in the cells and tissues represent a clear medical need and important research tools. Assays designed with this aim are always in development for research and for the translation to bioengineered kit for clinical biochemistry. In the field of biomarker search, in particular, the detection of the protein levels is essential to monitor its overexpression linked to pathological status or drug resistance events. This is a challenging field due to the lack of specific assays. In our case studies, we designed a sensitive assay to detect the levels (200-400nM) of the protein Thymidylate synthase (*h*TS) enzyme (both dimer and monomer) after treatment with different inhibitors, which allows to take an actual 'intracellular snapshot' of the *h*TS equilibrium. *h*TS is a widely used/proposed biomarkers in colorectal, lung or pancreatic cancer prognosis and a pharmacological target of anticancer therapeutic agents such as 5fluoruracil [1]. Drug resistance related to *h*TS overexpression results in chemotherapeutic failure induced by the dimeric form of the enzyme (active) with respect to the *h*TS monomer, inactive and rapidly degraded by the proteasome [2]. Therefore, it is a good example to propose a newly conceived a quantitative assay that include both dimer and monomer forms and allow the detection of the functional protein status. To this aim, we used as a starting model, the engineered HEK293 cells ectopically expressing wild-type *h*TS and hTS-F59A dimer interface mutant, labelled with Myc-DDK flag (Kd=40nM and > 10µM, respectively). To realize the model, we administered to the cells one selected chemical probe developed in our laboratories, and MG132 was used to prevent *h*TS proteasomal degradation. The subsequent addition of bis(sulfosuccinimidyl)suberate (BS3), a homobifunctional cross-linking reagent, permitted the capture of the actual amount of intracellular *h*TS, both monomer and dimer. Cell lysates underwent Western blot protocol. The results highlight the reduction of the dimer-monomer ratio of wt-*h*TS upon exposure to the probe with respect to control. Also, hTS-F59A mutant, nearly exclusively present in a monomeric form, was not detectable in its dimeric conformation, corroborating the sensitivity of the assay. Due to the low costs compared to E.L.I.S.A, and its unique ability to take an actual frame of the intracellular molecular equilibrium, further studies could be engaged to build a high throughput version of this tool. This strategy can be applied to other proteins and can be optimized and validated through the identification of the suitable limit of detection and cut-off for a diagnostic kit.



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Sustainable PBS-based biocomposite for 3D-printing

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Globally, a large amount of food is wasted and this represents a loss of resources[1]. The increasing consumption of single-use plastics is a serious issue too. In fact these plastics are not biodegradable but can be recycled, still offering good mechanical properties, good water and gas barrier properties. However, in most cases these materials are not recycled, accumulating in the environment, and the companies are pushing to find new solutions for a greater sustainability[2]. One solution to this problem may be the replacement of these non-biodegradable disposable materials with bio-composites that have the same functional characteristics and consist of biodegradable polymer matrices loaded with natural fillers, derived from food waste[3]. These bio-composites can be processed by conventional processing techniques.

In this work, a method is described for the preparation of a biodegradable composite material to produce innovative filaments suitable for fused deposition 3D printing (FDM), allowing the production of three-dimensional objects with complex geometry and tunable properties. The composite material consists of poly(butylene succinate) (PBS), as polymer matrix, reinforced with micro particles of titanium dioxide (TiO_2) at different concentrations, using soy lecithin (SL) as coupling agent. Composite pellets were prepared by solvent casting and then extruded with a co-rotating twin-screw extruder. Finally, the composite filaments were printed with an FDM printer and the effects of the printing parameters were studied on the obtained specimens.

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LISTA DEGLI ABSTRACT

Flash Communications

pag.	33	**FC14** **Togliatti Elena**	*Characterization of poly (butylene-adipate-terephthalate) – calcium-phosphate glass composites: viscoelastic properties and degradation*
pag.	21	**FC2** **Vezzoni Carlo Alberto**	*An argininocalix[4]arene for the effective delivery of miRNAs*
pag.	33	**FC16** **Volpi Stefano**	*Peptide nucleic acids – peptide conjugates as tools for targeting miRNA-mediated drug resistance in chronic myeloid leukemia*

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