DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

First STeBICeF Young Researcher Workshop

I giovani ricercatori del Dipartimento raccontano le loro ricerche alla comunità.

Book of abstract

Palermo
12 Gennaio 2023, ore 9:00
Viale delle Scienze, Ed.16
Aula Mutolo

Università degli Studi di Palermo

Premessa

Il Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF) ha un'anima multidisciplinare che può e deve rappresentare un punto di forza nel panorama scientifico dell'Università degli Studi di Palermo. Numerose linee di ricerca vengono perseguite in differenti ambiti e da parte di docenti e ricercatori appartenenti a vari settori scientifico-disciplinari.

"**First STeBICeF Young Researcher Workshop**" è un evento, promosso dal Direttore, Prof. Vincenzo Arizza, che nasce allo scopo di condividere, in una giornata dedicata alla ricerca scientifica, le varie esperienze ed i risultati raggiunti dai ricercatori del dipartimento.

I vari gruppi si confrontano con la presentazione di poster, mentre i ricercatori più giovani, che sono una preziosa risorsa per STEBICEF, illustrano e condividono i loro progetti e le loro ambizioni con delle presentazioni orali.

Il solo spirito scientifico è incompleto se non è accompagnato dalla volontà di condivisione delle nuove conoscenze acquisite.

Ci auguriamo che l'evento possa fornire numerosi spunti di future collaborazioni tra i vari gruppi di ricerca che mirino al conseguimento di risultati sempre più importanti.

Ad maiora...

A cura di:
Giuseppe Bazan, Calogero Fiorica, Annamaria Martorana, Maria A. Ragusa, Maria Luisa Saladino. dipartimento.stebicef@unipa.it

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Programma

9:00-9:30 Saluti istituzionali del *Direttore del Dipartimento STEBICEF: Vincenzo Arizza*

Plenary 1

Chairs: M. Labbozzetta e F.S. Palumbo

9:30-9:45 **O1**: *Alessandro Presentato*
Bacterial interaction and processing of chalcogen oxyanion: physiological and biotechnological aspects

9:45-10:00 **O2**: *Elena Piacenza*
Innovative, antimicrobial, bio- and eco-compatible coatings for orthopedic implants

10:00-10:15 **O3**: *Luca Vecchioni*
Fantastic Beasts and Where to Find Them: the "Biologia evoluzionistica e delle popolazioni" laboratory research group, activities and future perspective

10:15-10:30 **O4**: *Carla Rizzo*
Green Processes for synthesis and application of organic compounds and materials

10:30-10:45 **O5**: *Viviana Barra*
Chromosome Instability, friend or foe: genetic and epigenetic causes in cancer

10:45-11:05 **Coffee break**

Plenary 2

Chairs: P. Cancemi e F. Giacalone

11:05-11:20 **O6**: *Giulia Di Prima*
Development of novel secondary raw materials from grape juice fining waste as enriched excipients for oral care products and pharmaceuticals

11:20-11:35 **O7**: *Teresa Faddetta*
Multi-omics unveil molecular processes involved in plant-growth promoting activity of two selected actinobacteria on Solanum lycopersicum

11:35-11:50 **O8**: *Angelo Spinello*
In silico mechanistic investigation of biomolecular systems

11:50-12:05 **O9**: *Simona Campora*
Functionalization of PVP nanogels for tumour therapy

12:05-12:20 **O10**: *Vincenzo Campisciano*
Carbon Nanoforms in Catalysis

12:20-14:20 **Sessione Poster e Pranzo**

Plenary 3

Chairs: B. Parrino e F. Palla

14:20-14:35 **O11**: *Antonietta Notaro*
Methyl gallate: story of a phytochemical stolen from plant kingdom as possible preventative or protective molecule for colon cancer treatment

14:35-14:50 **O12**: *Riccardo Bonsignore*
From Inorganic Medicinal Chemistry to Organometallic Catalysis: a 10-years journey

14:50-15:05 **O13**: *Manuela Mauro*
Anthropic impact, bioactive molecules, sustainable development, my keyword? Biodiversity!

15:05-15:20 **O14**: *Marina Massaro*
Modification of clay minerals for several applications

15:20-15:35 **O15**: *Simona Terzo*
Impact of Indicaxanthin from Opuntia ficus-indica Fruit on metabolic disorders and neurodegeneration in High-Fat-Diet-Fed Mice

15:35-15:55 **Coffee Break**

Plenary 4

Chairs: A. Terenzi e G. Avellone

15:55-16:10 **O16**: *Annalisa Martorana*
Green synthesis of silver nanoparticles for the production of antimicrobial microparticles and injectable polysaccharide-based-hydrogels

16:10-16:25 **O17**: *Roberta Flavia Chiavetta*
Selective HCT116 cancer cells elimination by combining CDK1 depletion, senescence induction and treatment with senolytic natural drugs

16:25-16:40 **O18**: *Francesco Armetta*
Spectroscopy for the characterization of materials and cultural heritage

16:40-16:55 **O19**: *Gaia Pucci e Clara Cardella*
Zebrafish-based evaluation of innovative therapies in the fields of radiobiology and regenerative medicine

16:55-17:10 **O20**: *Fiammetta Pantò*
How to create a startup

17:10-17:20 *Antonio Palumbo Piccionello*
Premiazione vincitori Premio Ricerc@STEBICEF 2022:
S.E. Drago; D. Carbone; T. Faddetta; T. Miclot

17:20-17:30 *Direttore STEBICEF: Vincenzo Arizza*
Conclusioni

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Bacterial interaction and processing of chalcogen oxyanion: physiological and biotechnological aspects

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The chalcogens selenium (Se) and tellurium (Te) are rare Earth elements widely applied for the manufacturing of different devices expandable in a variety of application fields (i.e., electronics, biomedicine, and renewable energy, to name a few)[1]. The intense use of Te- and Se-containing compounds, and the improper disposal of the corresponding waste, led to the accumulation of these chemical species in almost every environmental niche. Among chalcogen compounds, oxyanions tellurite (TeO_3^{2-}) and selenite (SeO_3^{2-}) are the most soluble, bioavailable, and toxic[2]. Also, the low abundance of the mineral forms of such elements determines that their natural supply will end shortly with possible economic and technological effects. Thus, Te- and Se-containing waste represents the source from which these elements should be recycled and recovered. Microbial biotransformation of oxyanions into less toxic chalcogen species is the most appropriate concerning the circular economy[3]. Among bacterial genera, Actinomycetes are ideal candidates in environmental biotechnology, although the exploration of their potential for oxyanion biotransformation is scarce due to limited knowledge regarding the microbial processing of these chemical species. Here, the present talk will highlight some physiological aspects concerning the cell tolerance, adaptation, and response to SeO_3^{2-} of a *Kitasatospora* strain isolated from soil devoted to farming. To this aim, an integrated biological and physical-chemical approach combining physiological and biochemical assays with scanning electron (SEM) microscopy was designed. *Kitasatospora* cells experiencing SeO_3^{2-} stress revealed a series of striking cell responses, such as cell morphology changes, extracellular polymeric substance production, cell membrane modifications, oxidative stress burst, and the production of selenium nanospheres as a product of SeO_3^{2-} detoxification. These results highlight this *Kitasatospora* strain as an asset for biotechnological purposes, accomplishing the remediation of selenite-contaminated environments and recovering selenium at the nanoscale form.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Innovative, antimicrobial, bio- and eco-compatible coatings for orthopedic implants

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Antimicrobial Resistance (AMR) refers to microorganisms' ability to persist antibiotics or biocides that will determine, by 2050, more than 10 million deaths and an economic toll comparable to the 2008 financial crisis[1]. AMR-related infections regarding orthopedic implants (OIs) are becoming more pressing threats[2,3]. To date, OIs' antimicrobial potential derives from adding late-generation antibiotics, metal ions, or nanomaterials (NMs)[2,3]. Yet, antibiotics are limitedly effective, metal formulations can be toxic for humans, and their production and disposal have a high environmental and economic impact[2,3]. Besides, pathogens already developed AMR towards these antimicrobials[2]. Antimicrobial, bio-, and eco-compatible coatings for OIs (OICs) represent ideal alternatives to overcome these issues. This contribution focuses on the design and development of an innovative OIC composed of (i) selenium nanoparticles (SeNPs) and (ii) citrus essential oil (COE) as antimicrobial agents, (iii) hydroxyapatite nanowires (HAPNWs) as an osteointegration element, and (iv) polymeric films. SeNPs were produced through a biological route or a new green chemical synthesis in a confined environment to limit NP size and confer thermodynamic stability. The SeNP antimicrobial efficacy was implemented by loading different amounts of COE (SeNPs@COE), a natural biocide whose hydrophobic nature usually impairs its antimicrobial activity. HAPNWs were obtained by optimizing a green solvothermal procedure, even using eggshell waste as a raw material. Subsequently, new nanoformulations containing HAPNWs and SeNPs@COE (HAPNWs@SeNPs@COE) were generated. Biocompatible films were obtained by using chitosan or pectin as natural polysaccharides. Chitosan-based films were then loaded with SeNPs or the natural biocide curcumin while, following the circular economy principle, we recovered and revitalized citrus peel waste to obtain pectin-based films. Both films and nanoformulations were characterized from a physical-chemical perspective to gain information regarding their structure and intrinsic features. Moreover, pectin-based films efficaciously inhibited the growth of Gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains. On the opposite, formulations and films containing SeNPs, COE, and curcumin were more active against the Gram-positive *Staphylococcus aureus*, the principal pathogen responsible for OI infections.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Fantastic Beasts and Where to Find Them: the "Biologia evoluzionistica e delle popolazioni" laboratory research group, activities and future perspective

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The research group of the "Biologia evoluzionistica e delle popolazioni" laboratory is mainly focused on studying the systematics and phylogeography of marine, terrestrial and freshwater organisms. The research carried out in recent years has focused mainly on the characterization of the freshwater invertebrate fauna of the Palearctic biogeographical region, specifically of the central Mediterranean countries, implementing an integrative morphological and molecular approach. Such methodologies have allowed us to characterize in detail, the studied taxa and populations and to resolve uncertain taxonomic statuses and nomenclatural issues. As a result of such an integrated taxonomical approach, we shed light on the evolution, phylogeny and phylogeography of several taxa, among which marine and terrestrial turtles, fish and crustacean species. Recently we were able to approach the *Metabarcode* and the "*Next Generation Sequencing*", allowing us to explore the biological diversity of the studied habitats with greater detail. Finally, in addition to the research lines mentioned above, particular attention is devoted to the study of the phenomenon of biological invasions, which are known to be one of the greatest threats to biodiversity conservation at a worldwide scale.

Green Processes for synthesis and application of organic compounds and materials

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Sustainability of synthetic chemistry processes and the application of eco-sustainable materials in a wide range of applications are hot topic of research.
In this context, organic salts formed by organic cation and anions, thanks to their wide versatility can been used in dependence of their physicochemical properties in several fields. In particular, the combination of cation and anion can give rise to Ionic Liquids (ILs) if they present melting temperatures below 100 °C. ILs have been extensively applied as eco-sustainable solvents thanks to their negligible vapor pressure, volatility and flammability. Recently ILs have been applied for the synthesis of industrial interest products from biomass matrices.[1]
On the other hand, organic salts presenting structural functionalities that allow the occurrence of self-assembly processes can give rise to gelators or ionic liquid crystals (ILCs). Gelators have been used to form supramolecular gels in water, organic solvents, ILs or deep eutectic solvents. The corresponding gels can be used for environmental remediation and prevention as well as for biological application.[2] While ILCs can be used as alternative materials for smart and energetic devices.[3] Finally, the increasing interest in the conversion of biomass waste in products of industrial value can bring to the transformation of biomass in biopolymers. In all cases, a particular attention will be devoted to the use of alternative synthetic methodologies such as microwave or ultrasound irradiation as well as photochemistry techniques.

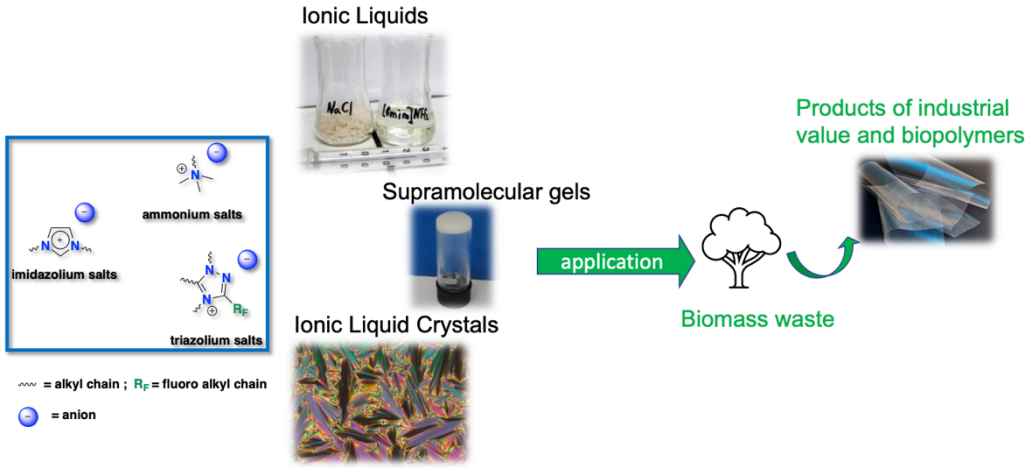

Figure 1. Schematic representation of organic compounds, materials and applications of interest.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Chromosome Instability, friend or foe: genetic and epigenetic causes in cancer

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Chromosome Instability (CI) compromises the fidelity of the transmission of the genetic material, and thus, is a grave risk for health. Many genetic diseases including cancer are characterised by CI. However, the leading causes identified so far can be different in each condition. Understanding the specific underlying mechanisms and how cells learn to deal with CI is of great relevance to design *ad hoc* and personalised therapeutic strategies.
With this aim, we have been focused on three research lines: 1) investigating the involvement of DNA methylation in centromere stability and function, 2) unveiling a new player in chromosomal common fragile sites (CFSs) stability, 3) exploiting CI to induce senescence and allow cancer cells' clearance.
By using molecular and cell biology approaches, as well as microscopy techniques, we observed that global DNA methylation loss, a frequent event in cancer cells, undermines the correct loading of centromere proteins resulting in mitotic defects and CI. We also identified a key element, an helicase protein involved in the resolution of RNA:DNA hybrids at CFSs during mitosis, that appears to be critical for cancer cells' survival. Moreover, we showed that curcumin treatment leads to senescence only tumor cells by increasing their endogenous CI. We also demonstrated that the combination of curcumin treatment and senolytic molecules is able to deplete cancer cells [1].
Our results pave the way for new therapeutic strategies against cancer progression that take advantage of the CI distinctive for each tumor context. We also set the course for DNA methylation essential to maintain centromere functionality and thus chromosomal stability.

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Development of novel secondary raw materials from grape juice fining waste as enriched excipients for oral care products and pharmaceuticals

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Nowadays, great importance is given to the waste products and particularly to their fate to found novel smart strategies capable of reducing their environmental footprint. Particularly, the waste products obtained by the grape processing industries are actually under the spotlight as they could be valuable sources of functional molecules as polyphenols. Stalks, pomace and wine lees are considered the main source for the recovery of polyphenols, while to date the waste bentonite has never been considered as a precious waste still rich in functional substances yet. Bentonite is an inorganic fining agent greatly able to remove protein haze permitting to clarify musts and wines and because of this largely preferred by the winemaker industries. In an ever-growing perspective of circular economy, the bentonite, considered before as just an abundant waste (100 g of bentonite are used to fine 1 hL of must/wine), may still be a valuable source of polyphenols and could be further valorized by means of their extraction. In view of a waste-to-market approach, green and innovative extraction solvents, already known as excipients for pharmaceuticals and cosmetics, will be tested with the objective of producing novel enriched raw materials directly useful in several fields to benefit from the presence of functional polyphenols. This project aims at recovering the waste bentonite from the fining of biological grape musts by collaborating with a local grape processing company (Bono&Ditta S.p.A., Mazara del Vallo, TP). This waste will be evaluated as source of polyphenols by several extraction methods as well as several “green” extraction solvents both hydrophilic (e.g., propylene glycol, polyethyleneglycol, glycerin) and lipophilic (e.g., ethyl oleate, isopropyl myristate). The extracts will be characterized in terms of antioxidant power, total phenolic and protein contents, quantification of some representative polyphenols and cytocompatibility. The further collaboration with the partner EVRA S.R.L. SOCIETÀ BENEFIT (Lauria, PZ) will lead to scalable, standardized and high-quality raw materials. The goals of the project are both to obtain new “enriched” excipients as marketable products as well as to employ them for developing novel oral care products and/or medical devices useful as adjuvants in the treatment of several buccal diseases (e.g., oral lichen planus and mucositis). The already collected data highlighted the effectiveness of PEG200 and propylene glycol as innovative extraction solvents leading to colored extract characterized by relevant amounts of functional biomolecules and thus significant antioxidant power.

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Multi-omics unveil molecular processes involved in plant-growth promoting activity of two selected actinobacteria on *Solanum lycopersicum*

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Chemical fertilizers in agriculture practices resulted in environmental pollution with changes in soil ecology [1]. The use of plant growth-promoting bacteria (PGPB) as biofertilizers is considered an eco-friendly alternative due to low impact on human health and environment [2]. PGPB enhance the uptake of soil nutrients, abiotic stress tolerance, crop quality traits and increase rhizosphere fertility [2]. The development of innovative PGPB-based biofertilizers is the aim of BIAS project (PO-FESR Sicilia 2014-2020). Different potential PGPB were assayed for *i*) indoleacetic acid production, *ii*) organic and inorganic phosphate solubilization, *iii*) saline and drought stresses tolerance, *iv*) nitrogen fixation. Among the most interesting strains, *Streptomyces violaceoruber* and *Kocuria rhizophila* were analyzed for their secreted and cellular metabolome by HPLC-MS. These strains were also assayed to evaluate their effects on tomato plants (*Solanum lycopersicum*) that were cultivated in different experimental systems, spanning from filter paper in Petri discs to a real field crop. The two strains showed a positive effect on plant growth and development, particularly in improving rooting, flowering and fruiting. As revealed by global analyses of epigenetic markers, transcriptomics and proteomics, this effect is related to a global expression regulation of plant genes, some of them involved, for examples, in nitrogen metabolism and ATP metabolic processes. Thus, these results suggest the use of *S. violaceoruber* and *K. rhizophila* to develop novel biofertilizers based on PGPB activity.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

In silico mechanistic investigation of biomolecular systems

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Computational models mirroring real life applications have become pivotal for most advances made in chemical sciences nowadays, as testified by the Nobel prize in Chemistry awarded in 2013 to Martin Karplus**,** Michael Levitt and Arieh Warshel. During the last 10 years I have applied a broad set of computational techniques (e.g. docking, classical and QM/MM molecular dynamics, enhanced sampling and free energy methods), in close collaboration with national and international experimental groups: i) to investigate the structural impact of pathogenic mutations strictly involved in cancer onset [1]; ii) the catalytic mechanism of metalloenzymes [2]; iii) computer-aided drug design of novel small-molecule inhibitors (both organic molecules or metal complexes) targeting metalloproteins [3] or nucleic acids structures (e.g. G-quadruplex) [4].
These compounds, often designed on specific patient's genetic profile, targeting specific carcinogenic pathways, may be employed in calibrated precision medicine approaches.

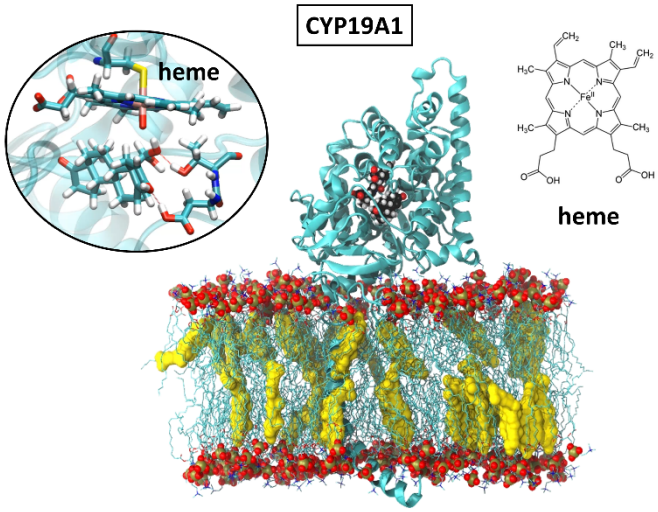

Figure. *The cytochrome P450 aromatase (CYP19A1) embedded in a membrane model. The inset shows the heme prosthetic moiety and the endogenous substrate androstenedione.*

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Functionalization of PVP nanogels for tumour therapy

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Cancer is one of the leading causes of death in the world. Over the past several decades, the development of engineered nanosystems for targeted drug delivery has received great attention thanks to their possibility to overcome the limitations of classical cancer chemotherapy including poor solubility, targeting incapability, nonspecific action and, consequently, systemic toxicity [1]. In this context, Polyvinylpyrrolidone (PVP) nanogels (NGs) were synthesized by e-beam irradiation that permits to obtain, in only one step of the synthesis, biocompatible, sterile and functionalized (with amino or carboxyl groups) NGs with a specific size. *In vitro* cell studies had demonstrated their biocompatibility and capability to be internalized by cells, while specific tumour targeting was obtained by conjugating folic acid or the antibody against integrin $\alpha_v\beta_3$. Moreover, nanogels were conjugated to a chemotherapeutic drug or to a pro-apoptotic siRNA through a glutathione-sensitive spacer, to obtain a controlled release mechanism, specific to cancer cells. Therefore, the possibility to release biological molecules in a controlled way (mediated by Glutathione) and recognize the specific tumour target allows for overcoming the typical limits of classic cancer therapy.

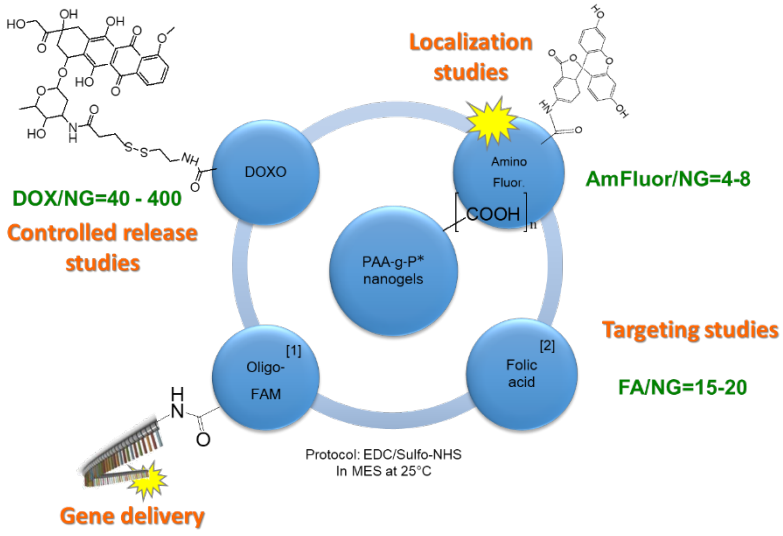

Figure. Functionalization of PVP nanogels for tumour therapy. .

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Carbon Nanoforms in Catalysis

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Carbon nanoforms (CNFs) have been extensively studied and applied in a plethora of applications as witnessed by their widespread use in different fields such as electronics, photonics, and nanomedicine. Furthermore, CNFs can be exploited as useful scaffolds for the preparation of nanostructured catalysts with high performances.[1] High chemical inertness under many conditions, thermal stability and mechanical resistance along with a lightness that other conventional materials cannot match are some of the useful features that distinguish such unique class of carbon allotropes. Over the years, our Research group has developed a series of CNF-based catalytic systems applied for different chemical transformation such as Pd-mediated C–C cross-coupling reactions, organocatalyzed C–C bond forming reactions, alcohols oxidation, and fixation of CO_2 into cyclic carbonates (Figure 1). The selected CNFs, namely fullerene C_{60}, single- and multi-walled carbon nanotubes, and carbon nanohorns, have been covalently functionalized by means of various approaches involving both polymerization of proper monomers onto their surface or precise functionalization to obtain specific molecular species. A review of some of our results concerning the use of CNFs in catalysis will be addressed.

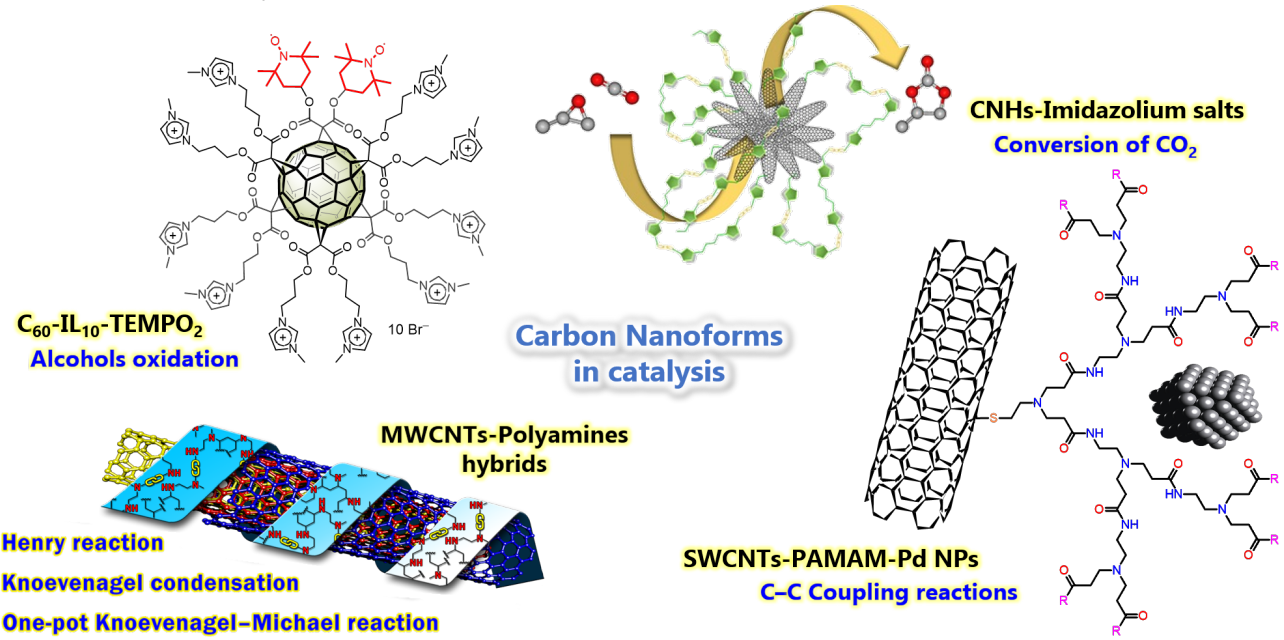

Figure 1. Graphical sketch of some CNF-based catalytic systems.

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Methyl gallate: story of a phytochemical stolen from plant kingdom as possible preventative or protective molecule for colon cancer treatment

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In the recent years, a growing interest has been turned towards bioactive compounds endowed with health-promoting properties. Indeed, the plant kingdom represents a rich source of phytochemicals that could find application in the cosmetic industry, in the nutraceutical research and, possibly, as adjuvants for anticancer therapies.
In this scenario, our research focused lately on *Mangifera indica* and *Litchi chinensis* [1,2], two tropical plants that found suitable pedoclimatic conditions for their spread in Mediterranean area some years ago. In particular, our studies focusing on mango fruit properties allowed to characterize the composition of extracts from different parts of the fruit, such as the seed coat (endocarp), seed and peel (exocarp). The analysis revealed that also the non-edible parts of the fruit, are an important bio-source of phenolic compounds, among these, methyl gallate, an ester of gallic acid, was one of the most represented.
Our studies provided evidence that methyl gallate exerts a strong cell viability inhibition of HCT116 colon cancer cells, while no cytotoxic effect occurs in differentiated Caco-2 cells, a model of intestinal cells. Interestingly, the use of methyl gallate on HCT116 cells reduces their viability by inducing an apoptotic pathway. Our results suggest that both oxidative stress and endoplasmic reticulum stress contribute to methyl gallate-induced cell death. In the early phases of treatment, the autophagic pathway is also activated, probably as a pro-survival response of the cells to the treatment. p53 seems to be the main player of the molecular mechanism induced by methyl gallate, since its expression increases into the nucleus and promotes caspase 3 activation and PARP fragmentation. Indeed, the methyl gallate-induced apoptosis is counteracted by interfering with p53 expression or with its transcriptional activity.
Our findings suggest that methyl gallate, a compound stolen from nature, could represent a candidate to use as valuable co-adjuvant in colon cancer treatment.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
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From Inorganic Medicinal Chemistry to Organometallic Catalysis: a 10-years journey

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During the last decades, metal complexes have occupied a pivotal role in medicinal chemistry as imaging or therapeutic agents[1]. While many of them were initially designed to interact with the right-handed DNA - following the example of the anticancer drug cisplatin – severe adverse effects triggered the need for more selective targets, such as cancer-related *i*) DNA structures or *ii*) proteins. On the one hand, numerous anticancer metal complexes have shown, for instance, potent stabilization of DNA G-quadruplexes (G4), non-canonical DNA structures involved in several biological processes, such as oncogene regulation[2]. On the other hand, metal-based compounds have found wide applications in protein targeting, often due to the intrinsic reactivity of the metal centre with their target's amino acids: cysteine arylation by Au(III) organometallic species have been, for instance, widely used to inhibit PARP-1 functions, still for anticancer purposes[3].
Overall, we will report on our advances in G4-targeting by small metal-based coordination and organometallics compounds. Furthermore, the application of Au(III) cyclometalated organometallic complexes in protein arylation and catalysis will also be discussed.

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Anthropic impact, bioactive molecules, sustainable development, my keyword? Biodiversity!

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Biodiversity is my main research focus and that of my research group. We study a range of different aspects concerning biodiversity, such as the impact anthropic activities have on biodiversity and how to improve its preservation, its use as a valuable resource for the extraction of bioactive molecules, and the study of its distribution. The research group has over twenty years of experience in the study of zoology, and, more in particular, the immune and behavioural responses of aquatic invertebrates and fish. For many years, the group has observed the effects of various anthropogenic activities, such as the acoustic and chemical impacts on the physiological and behavioral responses of invertebrate organisms, such as *Arbacia lixula* and *Mytilus galloprovincialis*, and of fishes, such as *Sparus aurata*, evaluating not only adult stage but also embryonic stage [1,2]. From a sustainable development perspective, we have recently turned our attention to an evaluation of the nutritional potential of aquaculture species which are recent additions to farming in Sicily: *Cherax quadricarinatus* and *Cherax destructor* [3]. However, biodiversity, as well as needing our protection, is also a resource which we can seek to harness in an eco-sustainable way. In recent years, research in our group has focused on the extraction of bioactive molecules from invertebrates and vertebrate living organisms (including from waste from the processing industry), with evident antimicrobial, anticancer and food preservation potential [4]. Last but not least, the regenerative capacities of invertebrate organisms such as *Holothuria tubulosa* [5] were evaluated with the possibility of transferring this knowledge to the biomedical sector. In addition to carrying out the aforementioned research, we are currently working on a nationally funded PON research project to conduct a freshwater biodiversity census in Palermo, including environmental DNA evaluation.

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Modification of clay minerals for several applications

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Clay minerals, are emerging materials which because of their interesting physico-chemical features and morphologies, have attracted considerable attention in the last few years. From a chemical point of view, their different chemical compositions allow to modify the clay surface opening the doorway to several strategies to tune the clay's properties. Specifically, it generates nanoarchitectures which have found application in several fields, ranging from biology to industry. Different kind of modification can be envisaged based on the supramolecular interactions of molecules or species with the clay surface or the grafting of specific functionalities.[1]
Herein we report the last results in the chemical manipulation of halloysite and hectorite surfaces to develop "smart" and multifunctional nanomaterials which have been used as drug carrier and delivery systems, for environmental remediation and as filler for polymeric matrices.
The advantage to modify the clay surfaces over the use of the pristine ones will be also highlighted.

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Impact of Indicaxanthin from *Opuntia ficus-indica* Fruit on metabolic disorders and neurodegeneration in High-Fat-Diet-Fed Mice

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Obesity is a metabolic disorder including diabetes, cardiovascular complications, hepatic dysfunction, and brain impairments. Since inflammation and oxidative stress, are linked to the development of metabolic diseases, dietary interventions promoting food rich in antioxidants and anti-inflammatory compounds can be helpful to prevent the onset of obesity-related dysfunctions. In light of this strict connection, the aim of the present study was to investigate the impact of Indicaxanthin (Ind), a bioavailable betalain pigment from *Opuntia ficus-indica* fruit, on metabolic disorders and neurodegeneration in an in vivo animal model with diet-induced obesity. C57BL/6J mice (n=24) were grouped as follows: 1. A group fed with a standard diet for 14 weeks (STD); 2. a group fed with a high-fat diet (HFD) for 14 weeks; 3. an Ind-group fed with HFD for 14 weeks, but it received Ind (HFD+Ind) per oral administration at a nutritionally relevant dose [1] of 0.86 mg/kg/day for the last 4 weeks. Biochemical, histological, immunohistochemical, western blotting and RT-PCR analyses were performed to assess metabolic parameters, liver, and adipose tissue inflammation and oxidative stress, peripheral insulin resistance, and neurodegeneration. Our results showed that Ind reduced the gain in body weight, daily food intake, and visceral fat mass, plasma fasting glucose and insulin concentrations, it improved glucose tolerance, insulin sensitivity, and HOMA-index. These effects were associated with reduction in hepatic and adipose tissue oxidative stress (RONS, malondialdehyde, NO levels, and number of inflammatory foci) and inflammatory markers (TNF-α, CCL-2, and F4-80 gene expression, p65, p-JNK, COX-2, and i-NOS protein levels and crown-like structures density). Finally, HFD+Ind mice showed a significantly lower number of apoptotic nuclei in the cerebral cortex, downregulation of Fas-L, Bim, and P27 (neuronal pro-apoptotic markers), upregulation of Bcl-2 and BDNF (anti-apoptotic factors) and lower levels of inflammation and oxidative stress than HFD mice, providing evidence for Ind neuroprotective effects. The present data suggest that Ind exerts beneficial effects in improving obesity-related metabolic disorders, including neurodegeneration in HFD mice. The effects appear to be mediated by reduction in peripheral and central oxidative stress and inflammation.

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Green synthesis of silver nanoparticles for the production of antimicrobial microparticles and injectable polysaccharide-based-hydrogels

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In the last years, silver nanoparticles (AgNPs), have been widely used as alternative antimicrobials and as a strategy to permit the relief of multi-drug resistance developed by bacteria [1].
Unfortunately, synthetic methods for the production of AgNPs involve the use of strong and not eco-friendly reducing agents. Green synthetic procedures have been set up using different natural biomaterials [2]. In particular, polysaccharides have been proposed as reducing and capping agents for AgNPs to develop nanocomposite antimicrobial biomedical devices. However, often chemical modifications help to improve nanoparticles stabilization.
Here, starting from hyaluronic acid and gellan gum, two natural anionic polysaccharides, we synthetized derivatives with pendant moieties able to induce in-situ production of AgNPs more efficiently than starting materials. In particular, a diethylene triamine derivative of hyaluronic acid (HA-DETA) [3] and a dopamine functionalized Gellan Gum (GG-DA) were characterized and used to obtain cytocompatible nanocomposite materials.
The specific chemical modifications endow the polysaccharides derivatives with physicochemical proprieties exploitable to produce smart biocompatible injectable hydrogels with dynamic pH-responsive bonds and self-healing biomaterials or to develop hydrogels loaded with Gellan Gum-ionic strength sensible nanocomposite microparticles. These multifunctional systems with environmental pH and ionic strength responses found different clinical applications, such as medical device coating and wound healing in infected injuries. Moreover, the development of these new AgNPs loaded biomaterials can permit a local and sustained release of silver ions, limiting toxicity to mammalian cells, usually associated with metal nanoparticles.

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Selective HCT116 cancer cells elimination by combining CDK1 depletion, senescence induction and treatment with senolytic natural drugs

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Uncontrolled cell proliferation is one of the main hallmarks of cancer. Unlimited cell division allows tumor cells to propagate even in presence of severe aberrations and, thus, is responsible for the cancer cells' great adaptive potential even in adverse environmental conditions. Cell cycle cyclin-dependent kinases (CDKs) have often been found to be altered in tumors, and, in particular, CDK1 is overexpressed in different cancer cell types. CDK1 is essential for cell cycle progression and its absence has been correlated with embryo lethality or cell death in normal cells. Due to its pivotal role in the cell cycle, its inhibition or depletion can potentially lead to a proliferation halt, especially in cancer cells. A prolonged cell cycle arrest can lead to cellular senescence, a cellular response to adverse triggers to limit the propagation of damaged cells.
For all these reasons, we decided to silence post-transcriptionally CDK1 in HCT116 cells in order to arrest the progression of the cell cycle. We observed cell cycle arrest in the G2/M phase, as well as the establishment of cellular senescence after three consecutive RNAi rounds targeting CDK1. Interestingly, the treatment of senescent cells with the senolytic drugs Quercetin and Fisetin, two natural molecules that specifically induce apoptosis in senescent cells, successfully removed the senescent cancer cells with respect to the control. Thus, combining CDK1 depletion for senescence induction with senolytic drugs can potentially be a powerful strategy for the selective elimination of cancer cells with overexpressed CDK1, paving the way for less invasive, more specific and natural anti-cancer treatments.

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Spectroscopy for the characterization of materials and cultural heritage

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The use of radiation or of particles as probes to investigate the matter by spectroscopic techniques is one of the main ways followed by researchers to define its structure and properties. Furthermore, there are several mechanisms of interaction able to provide indirect information about composition, bonds, and interactions, which describe the state of the system under investigation by proposing a proper model. For this reason, Spectroscopy can be considered a powerful tool for the study of materials by the characterization of the features influencing the properties. In the last years, the application field of spectroscopic techniques was extended from synthetic materials also to the study of goods of interests in the field of cultural heritage, more complex systems for variable composition, and less or absent prior knowledge, which requires non-invasively investigation as the main demand to preserve the integrity of the object. The chemical-physical analysis can reveal, for example, information about the production of the object (raw materials and technology of the manufacture), the aging (the chemical processes involved due to the interaction with the environment permanence in the time) and the conservation state (to tailor the actions in the restoration work or to preserve the object for future) [1]. There are some limits to the amount of information available from spectroscopy so it is necessary to develop approaches to standardize the setup and the methods for investigating a kind of object, extracting the highest amount of information with the lowest impact [2]. My research activity focused on that, developing new approaches devoted mainly to the study of archaeological metals and paintings. During the presentation, firstly a brief description of the research field will be provided, then some cases studios will be described in order to show the potentiality of spectroscopy applied to cultural heritage. One of the study regarding two Montefortino helmets [3] summarize how X-ray and neutron based spectroscopies permits to describe the metal of the goods from the production to the corrosion.

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Zebrafish-based evaluation of innovative therapies in the fields of radiobiology and regenerative medicine

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Cell cultures and small mammalian animals are well established widely-used models for preclinical investigations. However, in the era of rapid development of highly specific and selective treatments, there is a need for preclinical models that, with practical positive attributes as good reproduction captivity and easy laboratory care at a relatively low cost, could take into account the complex physiological phenomena related to organisms in their entirety. The zebrafish (*Danio rerio*) embryos fulfil these requirements, due to their small size, optical transparency, rapid development and similarity to humans in genetics and anatomy [1].
In our research group, we use zebrafish as a research platform to develop innovative therapeutic protocols in the fields of radiobiology and regenerative medicine. In fact, in the era of personalized therapy, radiotherapy could be used in combination with radiation modifying agents both to improve the therapeutic index and to personalize cancer treatment plans. In this *scenario* zebrafish embryo represents an excellent model, considering that embryogenesis is the most radiosensitive stage in the vertebrate life cycle and that the aqueous environment in which embryos develop favours homogeneity in the radiation dose distribution [2]. Similarly, zebrafish can faithfully reproduce pathological phenotypes, making it an excellent model in the field of regenerative medicine. In light of the limitations given by cell-based therapies in this field, and the finding that the main beneficial is due to paracrine effects of cell secretome [3], we focus on the therapeutic characterization of Conditioned Medium derived from Wharton's Jelly mesechymal stem cells. Our experimental workflows consist in the combined assessment of morphological, developmental, physiological, behavioral and molecular analysis of zebrafish embryos to evaluate the protective and side effects induced by innovative therapeutic treatments.

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How to create a startup

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As it has long been recognized by economists (Acs, Audretsch & Feldman, 1992) [1], research and the production system's innovative capacity are strictly linked and an active collaboration between these two worlds leads to a phenomenon defined as "technological transfer".
Technology transfer includes all those activities that underlie the passage of a series of factors (including knowledge, technology, skills, manufacturing methods, production standards and services) from the scope of scientific research to the market one.
University spin-offs are potential producers of positive and beneficial externalities not only for the academic environment, but above all for the socio-economic context of reference.
The legislative interventions of the last twenty years, operating on several aspects (university reform, PA decentralization, local autonomy, local economies strengthening), have led to a new regulatory system, able to structuring an environment suitable for the economic exploitation of research results by academic institutions, contributing to the professionalisation of scientific activities and encouraging new investment processes in forms of academic entrepreneurship.
But any business idea, no matter how brilliant, remains only an idea if it is not well developed so to become a profitable enterprise: a startup always born from an idea… from a sheet of paper full of notes, from a graphic project or from an intuition, but to obtain a successful startup it is necessary to implement a series of processes that follow one another and borrow from the common denominator of the basic idea.
A clear definition of the path to follow to transform the idea into a startup is the best starting point.

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In vitro effects of extracts from leaves and rhizomes of *P.oceanica* on HepG2 tumor cells

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Bioactive compounds produced by aquatic species exhibit a wide range of therapeutic effects in humans and represent promising prevention and/or treatment agents and beneficial supplements for the formulation of functional food and food-packaging material.[1-2] In order to identify novel potential anti-tumoral substances, aqueous extracts from green (GLE) and beached leaves (BLE) and rhizomes (RE) of the marine seagrass *P.oceanica* were tested on HepG2 hepatocarcinoma (HC) cells to study cell viability/proliferation, cell cycle, apoptosis and autophagy modulation, mitochondrial function and redox state.[3] GLE and RE, but not BLE that was not tested further, affected cell viability in a dose-response manner and the IC_{50} at 24h was calculated and used in the subsequent assays. Cell cycle impairment and the accumulation of Annexin-V^+/PI^- cells at early times of exposure indicated the apoptosis-promoting effect of both extracts, as also proven by the detection of a panel of activated caspases. The intracellular accumulation of acidic vesicular organelles, hallmarks of autophagy, decreased after both treatments, more drastically after exposure to RE which also induced the loss of mitochondrial transmembrane potential. Notably, viability inhibition was not reverted by co-treatment of RE with the autophagy-stimulator rapamycin, confirming a more extensive cell damage than mere autophagy inhibition. Real time-PCR and Western blot analyses were also performed to check the expression levels of genes and the accumulation of proteins related to the apoptotic and autophagic processes upon treatments. Moreover, only GLE induced the steady downregulation of intracellular ROS. Consistent with a potential suppression of HC metastatic attitude, treatments with both extracts also induced an early block of cell motility in wound healing assays. Overall, the results obtained suggest the potential and diversified anti-HC ability of extracts from *P.oceanica* which appears to be stronger for RE than GLE and merits further investigation to identify the substance(s) responsible for the cytotoxic effect and to open new interesting scenarios for future biomedical and nutraceutical applications.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

A comparison of the anti-proliferative effects induced by Hydro-alcoholic or Supercritical Fluid Extraction from Sicilian vinification by-products on MDA-MB231 breast cancer cells

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Nowadays, different cancer types need of a number of treatment protocols in function of their severity. Therefore, the identification of new natural molecules to employ in combination with conventional anticancer drugs could improve therapy efficiency [1]. Most of natural compounds derive from edible part of vegetables and fruits, but in the last years, an increasing interest is shown for phytochemical molecules derived from agricultural wastes and by-products. The choice to use by-products could reduce environmental pollution derived from their disposal and it could be an inexpensive alternative in accord to circular economy, based on recycling and valorization.

With this in mind, in this study we are interested to isolate new phytochemical compounds from agri-food waste employing Supercritical Fluid (SF) Extraction, an eco-friendly method. This procedure uses CO_2 in supercritical state as solvent which ensures minimal alteration of the natural matrix and offers a number of advantages when compared to conventional hydro-alcoholic extraction method [2].

In preliminary study, we analyzed the anti-cancer potential of hydro-alcoholic and SF extracts from grape pomace, the solid residue generated from vinification process on MDA-MB231 breast cancer cells. Folin Ciocalteu method for the assessment of polyphenols concentration revealed a greater polyphenolics content in the hydro-alcoholic extract than in the SF one. Nevertheless, despite the lower polyphenols content, SF extract was much more active on MDA-MB231 cells. Indeed, we obtained the same viability reduction (about -70%) employing 12,5 μg/ml GAE of SF extract *vs* 150 μg/ml GAE of hydro-alcoholic extract.

This result seems to underline a different composition of the two extracts in spite of coming from the same matrix. It is still a preliminary study, the next step will be the chemical characterization of the extracts.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Biological activities of the extracts from macroalgae *Carpodesmia crinita*, *Carpodesmia brachycarpa*, *Asparagopsis taxiformis*

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Marine species represent a rich source of biologically active products that can be used in various fields. Among them, marine algae produce numerous secondary metabolites responsible for different biological activities such as: immunomodulatory [1], antioxidant [2], and antimicrobial [3]. The aim of this study was chemically characterizing the extracts of three macroalgae species: *Carpodesmia crinite (Duby)* Orellana & Sansón, 2019, *Carpodesmia brachycarpa* (J. Agardh) Orellana & Sansón 2019, *Asparagopsis taxiformis* (Delile) Trevisan 1845 and evaluate their biological activities. The characterization of the secondary metabolites was performed by HPLC-MS and the results obtained showed higher meroterpenoids levels. Moreover, the extracts tested against the *Arbacia lixula* sea urchin modulate the total and differential cellular count demonstrating their involvement in immunity responses. Furthermore, important antimicrobial activities were observed by testing these extracts against the bacterial strains *Listeria monocytogenes* and *Staphylococcus aureus*. For the first time our study shows the effects of macroalgae extracts on the immunomodulatory activity in *Arbacia lixula* sea urchin and important antimicrobial activity. The results obtained, although preliminary, are certainly encouraging and our purpose is also improved this information performing biochemical and molecular assays of extracts obtained to understand better the potential that these metabolites have towards the sea urchin *Arbacia lixula* that graze on these macroalga.

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FUNCTIONAL RESCUE OF F508del-CFTR USING SMALL NITROGEN HETEROCYCLES

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Cystic fibrosis (CF) is a recessive genetic disease found primarily in Caucasians and caused by mutations that impair the function of the CFTR chloride channel. Among the 2000 known CF mutations, deletion of phenylalanine at position 508 (F508del) is the most common one, causing multiple folding and stability defects in CFTR protein which result in premature degradation.[1] Pharmacological correction of mutant CFTR defects is an effective therapeutic strategy for CF. In particular, combinations of correctors with complementary mechanisms can be used to maximize the rescue of F508del-CFTR protein.[2] We have recently identified a very promising class of small molecules, PP compounds, in the rescue of F508del-CFTR in cell lines and in primary airway epithelial cells, particularly in combination with type 1 correctors such as VX-809. Very interesting results emerged for compound PP28 that elicited a strong synergism when combined with VX-809. A translational approach based on multidisciplinary studies is now driving our efforts to generate more effective and potent analogues as useful tool for precision medicine in CF. Several iterative cycles of chemical synthesis and evaluation of the corrector activity have provided so far useful information about the structure-activity relationship (SAR) of the chemical entities synthetized. Some new potent analogues emerged as F508del-CFTR correctors, producing a rescue comparable to that of VX-809 and a strong synergism when used in combination with it. The pharmacological insight indicates that PP compounds possibly act as class 3 correctors. The optimization process of ADME profile is ongoing. We aim to obtain the best trade-off between potency/efficacy and “drug-likeness” in order to develop an optimized lead compound that could be considered for preclinical and clinical development.

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Triazole-based compounds as potential anti SARS-CoV-2 agents targeting Nsp13

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The lack of effective therapy against infections caused by SARS-CoV-2 still represents an unmet medical need. One of the most attractive targets for the development of new antiviral agents is the non-structural protein 13 (NSP13), due to its high degree of conservation and to its essential role in SARS-CoV-2 replication [1-2]. From a virtual screening of an *in-house* library of triazole-based derivatives on SARS-CoV-2 Nsp13 active site (PDB code: 7NNG) [3], an interesting compound (**1**) emerged due to its highly superimposable binding mode to that of the co-crystallized ligand (**Fig. 1**).

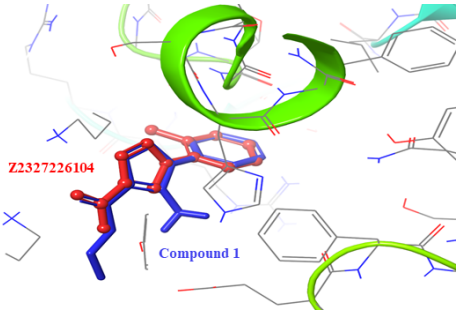

Figure 1. *Binding mode of the co-crystallized ligand (red) and compound* ***1*** *(blue).*

Based on these results, a series of [1,2,3]triazolo[4,5-*b*]pyridine and [1,2,3]triazolo[4,5-*h*][1,6]naphthyridine derivatives was designed and synthesized to explore the chemical space around the *hit* candidate. *In vitro* assays on SARS-CoV-2 infected cell cultures revealed a promising derivative with antiviral activity at micromolar level. The docking experiments followed by MM/GBSA analysis showed that this compound was able to establish some favorable interactions within the helicase binding site while also stabilizing the enzyme.

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Mechanism of Action (MOA) of novel TRIDs: tryptophan tRNA-specific 2'-O-methyltransferase FTSJ1 as likely pharmacological target to exert PTCs readthrough on CFTR mRNA

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Cystic Fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the CFTR gene, coding for the CFTR chloride channel[1]. About 10 % of the mutations affecting the CFTR gene are "stop" mutations, which generate a Premature Termination Codon (PTC), thus resulting in the synthesis of a truncated CFTR protein[1]. A way to bypass PTC relies on ribosome readthrough, that is the capacity of the ribosome to skip a PTC, thus generating a full-length protein[2]. "TRIDs" are molecules exerting ribosome readthrough and for some of them the mechanism of action is still under debate[2]. By in silico analysis as well as in vitro studies, we investigate a possible mechanism of action (MOA) by which our recently synthesized TRIDs, namely NV848, NV914 and NV930[3], could exert their readthrough activity. Our results suggest a likely inhibition of FTSJ1, a tryptophan tRNA-specific 2'-O-methyltransferase[4]. In addition, we report that our TRIDs do not exert readthrough on natural termination codons.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Proteolytic activity and MMP-14-like protein levels are affected by Vanadium in *Paracentrotus lividus* Embryo

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The increasing industrial use of vanadium (V), as well as its recent medical use in various pathologies has intensified its environmental release, making it an emerging pollutant [1]. The sea urchin embryo has long been used to study the effects induced by metals, including V. In this study we used an integrated approach that correlates the biological effects on embryo development with proteolytic activities of gelatinases that could better reflect any metal induced imbalances. V-exposure caused morphological/morphometric aberrations, mainly concerning the correct distribution of embryonic cells, the development of the skeleton and the embryo volume [2]. Moreover, V induced a concentration change in all the gelatinases expressed during embryo development and a reduction in their total proteolytic activity. The presence of three MMPs-like gelatinases (MMP-2, -9 and -14) was also demonstrated and their levels depended on V-concentration. In particular, the MMP-14-like protein modified its expression level during embryo development in a time and dose dependent manner. This enzyme also showed a specific localization on filopodia, suggesting that primary mesenchyme cells (PMCs) could be responsible for it synthesis. In conclusion, these results indicate that an integrated study among morphology/morphometry, proteolytic activity and MMP-14 expression constitutes an important response profile to V-action.

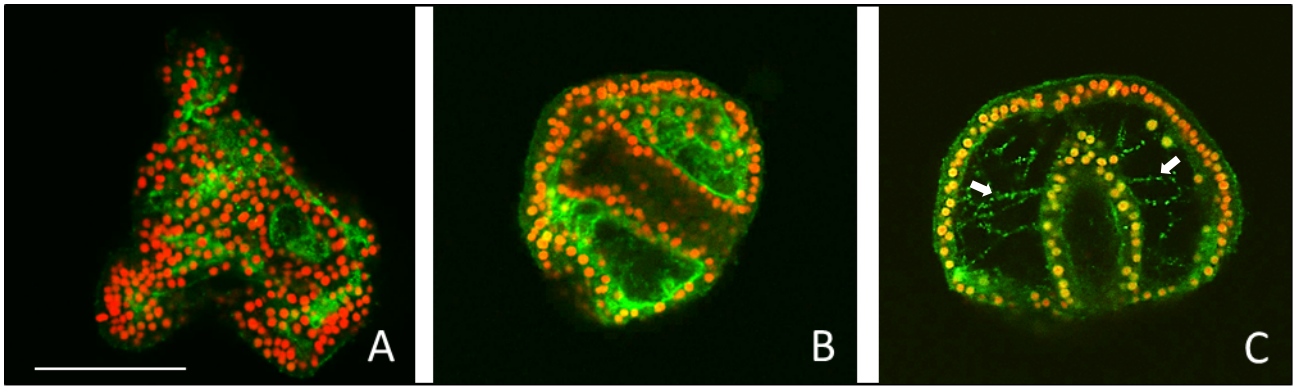

Figure. Immunolocalization analysis of MMP-14-like protease in whole mount embryos. Shown are representative confocal microscopy images of equatorial optical sections of embryos at 36 h of development/treatment. In green, MMP-14-like protein detection; In red, nuclei counterstaining with propidium iodide. (A) Control embryo; (B) 1 mM V-treated embryo; (C) 500 µM V-treated embryo. White arrows indicate the localization of MMP-14-like in filopodia. Scale bar = 45 µm.

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Combination of Clay Minerals for the development of smart drug carrier systems.

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Despite the progress of the pharmaceutical research, systemic chemotherapy has still to face major drawbacks such as limited drug selectivity, which results in cytotoxicity at the expense of healthy organs, and development of drug resistance, eventually affecting the chance of a total recovery. Drug penetration in solid tumors, on the other hand, is difficult, owing to barriers and poorly organized vasculature, with the possibility of sub-therapeutic treatment exposure and, as a result, reduced drug effectiveness.
The use of functionalized natural clay minerals, thanks to their morphologies and physico-chemical characteristics, are quickly becoming the focus of investigation offering improved bioavailability, dose-response and targeting efficiency, with reduced toxicity, therefore fewer side effects [1]. Moreover, clay minerals represent an optimal starting material for innovative therapies such as modified release, co-delivery et al., and gene chemotherapy[2-4].
Herein we report preliminary results of the development of smart nanomaterials based on the combination of halloysite and laponite for cancer treatment. In particular halloysite was used as carrier for epirubicin and methotrexate molecules; successively the obtained nanomaterials were added to laponite hydrogels to investigate their potential applications for the local future treatment of bladder cancer therapy.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
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Effects of the essential oil from a Sicilian accession of *Prangos ferulacea* in rat intestinal smooth muscle

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Apiaceae family, including *Prangos ferulacea* is a rich source of essential oils, highly valuable for medicinal or industrial purposes. A recent study (1) reported the chemical composition of the essential oil from an unexplored accession of aerial parts of *P. ferulacea* (Pangroil), growing wild in Sicily, highlighting its biological potential. This study aims to analyze the effects of Pangroil and of its main component, β–ocimen, on the contractility of rat small and large intestine. Mechanical responses to Pangroil and β–ocimen were examined *in vitro* as changes in isometric tension in the intestinal preparations.

Pangroil (0.012–0.2 mg/ml) in rat duodenal smooth muscle induced a dual response. At low concentrations, Pangroil induced a transient contraction, that decreased in amplitude by increasing concentration, followed by a muscular relaxation. At the higher concentrations, Pangroil induced only a significant dose-dependent muscular relaxation. In colonic muscle, Pangroil (0.012–0.2 mg/ml) induced only inhibitory effects, consisting in a reduction in the amplitude and frequency of phasic contractions and at the higher doses a decrease in basal muscle tone. Duodenal excitatory effects of Pangroil were abolished by ω–conotoxin, neural N-type Ca^{2+} channels blocker or atropine, muscarinic receptor antagonist. Instead inhibitory effects observed in both intestinal preparations were not affected by ω-conotoxin, L-NAME, NO synthase blocker or TEA, non-selective K^+ channel inhibitor. β–ocimen (0.007–0.12 mg/ml), the main component of Pangroil, induced in both preparations inhibitory responses, being less potent and efficient of Pangroil. Both Pangroil and β–ocimen inhibited the external Ca^{2+} influx- induced contraction, and shifted to the right contraction the response-curves to KCl and to carbachol, a cholinergic agonist.

Results indicate that Pangroil affects rat intestinal contractility with regional differences between small and large intestinal preparations. In small intestinal preparations it induces both contractile and relaxant responses, whilst only relaxation was observed in large intestinal preparations. Contractile effects are related to Achreleased by enteric cholinergic neurons. The inhibitory effects seems to be due to the blockade of extracellular Ca^{2+} influx and the reduction of Ca^{2+} release from the intracellular store in the muscle cells. β–ocimen, the main component of the Pangroil, is responsible, at least in part, for the observed spasmolytic effects. These studies encourage further research to provide whether Pangroil may have clinical benefits for treatment of gastrointestinal motor disorders.

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Bottom-up and top-down approaches for the synthesis of nanomaterials based on halloysite and carbon dots

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Halloysite is a natural clay mineral with a general formula of $Al_2Si_2O_5(OH)_4{\cdot}nH_2O$ and a predominantly hollow tubular structure (HNTs). It presents an external surface composed by siloxane groups and an inner lumen constituted by aluminum hydroxide. Due to this different composition HNTs possess a negative charge on the external surface and a positively charged lumen. Owing to the high mechanical strength and good biocompatibility, HNTs represent a versatile core structure for the design of functional nanosystems of potential technological and biomedical interest.[1] Recently it was reported the modification of halloysite with fluorescent molecules allowed the possibility to tune the physico-chemical properties both of HNTs and the chromophores.[2] In particular, the introduction of carbon dots on the external surface of halloysite, by a bottom-up strategy, led to the formation of photoluminescent halloysite based nanomaterials.[3] Herein, we report the synthesis of several nanomaterials based on halloysite and carbon dots, using two different approaches. The bottom-up approach allowed to form the carbon dots directly on the surface of the HNTs using as carbon source different dicarboxylic acids previously linked onto HNTs and as passivant agents three different amines, and the top-down approach where free carbon dots previously synthetized were linked on the external surface of modified HNTs. The nanomaterials obtained were thoroughly characterized by a physico-chemical point of view by FT-IR and thermogravimetric analysis, and their photoluminescence properties were investigated in solution and in solid state as well. Their morphology was investigated by transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS), the aqueous mobility by dynamic light scattering measurements. Furthermore, the nanomaterials antioxidant properties were also evaluated by means of the DPPH method.

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UNRAVELLING THE RELATIONSHIPS BETWEEN THE MICROBIOTA AND THE ENVIRONMENT

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Microorganisms play a critical role in the health of host organisms. When this correct balance, known as eubiosis, is disrupted due to various perturbations, we have dysbiosis. The microbial community can live and colonize several environments. The study of microbial communities in different systems, including humans, animals, and plants, has increased thanks to new technologies based on Next Generation Sequencing (NGS). Indeed, better microbiota knowledge can contribute to improving how these systems are interconnected and interact with each other. While each organism's microbiota is unique, some bacterial species are shared. Microorganisms are an essential component in ecosystems and have a significant environmental impact. As a result, a better understanding of the environmental microbiota may be critical to dealing with the world we live in. Meta-barcoding technology, which is based on NGS, enables the sequencing of a single gene, such as 16S rDNA, which is the gold standard for prokaryotic taxonomic and phylogenetic analysis. This type of analysis allows researchers to determine the abundance and diversity of microbes in various matrices, as well as their relationships. Given the fundamental nature of microbial life and diversity concerning host organisms and vice versa, microbiota research provides an interdisciplinary platform for many fields, including human medicine, food science, biotechnology, agriculture, ecology, and animal health.

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Translational Readthrough Inducing Drugs (TRIDs) induce the recovery of LPS Responsive Beige-Like Anchor (LRBA) protein expression in in vitro model characterized by the c.5047 C>T (R1683*)

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Nonsense mutations affect about 11% of the inherited diseases. They are due to single-nucleotide substitutions in gene codifying sequences, leading to the creation of premature termination codons (PTC) in the mRNA, and causing the production of truncated and non-functional proteins1. The suppression therapy, by readthrough of the stop codon, is one of the proposed approaches to restore protein expression2. In this work the ability of three new optimized TRIDs (NV848, NV914, NV930)3 to rescue the expression of the LRBA protein in LRBA (c.5047 C>T, R1683X) primary human fibroblasts has been evaluated. Since it is known that the readthrough either basal or induced by TRIDs allows the insertion of Trp, Arg, or Cys in the place of a premature stop codon UGA4, the possible aminoacidic substitutions were analyzed by structure assembly simulations to determine the influence on the protein structure. Finally, by NGS analysis we evaluated the correct mRNA sequence after TRIDs treatment to understand a possible interference of the molecules with the fidelity of the transcription process.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Spatial and temporal dynamics of microbial communities in an aquifer contaminated by chlorinated solvents

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Chlorinated solvents are widely spread, recalcitrant environmental pollutants. In contaminated aquifers, they are generally biodegraded by OrganoHalide Respiring Bacteria (OHRB) that reduce them in the anaerobic dehalorespiration process [1]. However, aerobic direct and cometabolic oxidative processes can also co-exist [2]. In order to define the microbial composition of an aquifer chronically and heavily contaminated by chlorinated solvents, never microbiologically characterized before, here we studied the groundwater autochthonous microbial communities and monitored their changes over time. The main chlorinated contaminant detected in the aquifer is 1,2-dichloroethane (1,2-DCA), whose concentrations undergo high fluctuations over time and have exceeded one gram per liter. Groundwater samples were collected from eight piezometers placed in the study area and were analyzed for microbial diversity by Automated Ribosomal Intergenic Spacer Analysis (ARISA) and for taxonomic profiling by 16S rRNA gene metagenomic sequencing. Autochthonous microbial communities were unexpectedly characterized by very high alpha and beta diversity. Among the identified taxa, chemolithotrophic, methylotrophic, sulphate-reducing and sulfur-oxidizing bacteria were revealed, while the relative abundance of known dechlorinating anaerobic and aerobic bacteria was very low. However, a large part of most groundwater communities was made up of unclassified taxa. Groundwater from the most 1,2-DCA contaminated area was sampled in three moments over two years and 16S rRNA gene Illumina sequencing was applied on metagenomic DNA from each sampling event. The microbial characterization revealed a reduction in bacterial diversity and an enrichment in Helicobacteraceae and Desulfuromonadaceae families over time. The presence of the dehalorespiring genera *Dehalococcoides*, *Dehalogenimonas* and *Desulfuromonas* was also PCR-detected using phylogenetic biomarkers. In conclusion, the low relative abundance of known anaerobic or aerobic dechlorinating taxa suggests a poor intrinsic biodegradation potential, consistent with the deep and chronic contamination at the site. However, this limited dechlorinating potential of groundwater could be exploited in bioremediation interventions, if properly biostimulated.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Biological responses to high-frequency sound in a freshwater crayfish, *Cherax quadricarinatus*

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Anthropogenic noise is recognised as an important environmental stressor that can have long-term negative consequences on species. In recent years, there has been increasing attention to the potential negative impact of noise pollution on species, with great concern for the importance of impacts on aquatic animal life.
This study examined the effects of acoustic stress on the biochemical parameters of the freshwater crayfish *Cherax quadricarinatus.*
The experiment was conducted in a tank equipped with an audio and video recording system using ten groups (five control and five test) of three adult crayfish (30 animals in total). The animals in the test group were exposed to acoustic signals [a linear sweep from 10 to 200 kHz lasting 1 s, with a sound pressure level between 138 and 157 dBrms (re 1lParms)] for 45 minutes. Biochemical parameters such as pH, osmolarity, protein concentration and enzyme activities (alkaline phosphatase, esterase and peroxidase) were evaluated. Enzyme activities show significant changes, with significantly lower values in stressed animals. These results suggest that high-frequency stimuli induce a physiological stress response, thus suggesting that acoustic stress may have physiological effects on the species.

Molecular strategies to investigate MBP-1 targets and restore high levels in cancer cells

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The Myc promoter binding protein -1 (MBP-1) is a 37 kDa protein synthesized from the alternative translation of ENO1 mRNA, encoding the α-enolase [1]. Both α-enolase and MBP-1 are involved in tumorigenesis, although as antagonists . ENO1 is involved in cell growth, hypoxia tolerance, and autoimmune activities besides its major role in the glycolytic pathway. On the contrary, MBP-1 suppresses cell proliferation and the invasive ability of cancer cells. MBP-1 acts in the nucleus as a transcriptional repressor of several proto-oncogenes, such as c-myc [3], HER2 [4], COX-2 [5], and FOXP3 [6]. In physiological conditions, the intracellular levels of MBP-1 are considerably lower compared to α-enolase because of their different translation efficiency and stability. However, in many cancers, including breast cancer, when α-enolase levels increase, a strong reduction of MBP-1 levels occurs [7].

Here, to elucidate the molecular pathways through which MBP-1 inhibits cell growth and increases MBP-1 expression in cancer cells, two different molecular strategies were employed.

The first one consists of transient and stable transfections of MBP-1 in breast cancer cells to identify the induced patterns of gene-expression modulations. Lipofectamine and the Tet-On 3G system were chosen for transient and stable transfections in cancer cell lines. Next, gene expression analysis will be performed by using MicroArray technology.

In the second approach, 4 oligonucleotides with imperfect complementarity have been designed to pair with ENO1 mRNA in the region containing the AUG in position 1, in order to block the canonical translation of α-enolase and promote the alternative translation of MBP-1. Two of them (ENOM1 and ENOM4), when transfected in SkBr3 breast cancer cell line, were able to increase MBP-1 levels compared to the controls. Next, to pass from a transient to a stable system, Knockout Single Vector inducible RNAi System will be used. The sequence encoding the shRNA precursors of ENOM1 and ENOM4 have been cloned under the control of the Tet-responsive Pol III hybrid promoter (PTight/U6).

In conclusion, the above molecular strategies, will provide new insights of the role of MBP-1 in tumorigenesis and will allow to develop a new therapeutic approach to restore the expression of alternatively translated genes.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Phytochemical Profile and Functional Properties of *Rubus idaeus* Seed Powder

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In the context of the contemporary research on sustainable development and circular economy, the quest for effective strategies aimed at minimizing food waste impact becomes more and more important. In particular, agri-food waste and by-products are still rich in bioactive compounds and could represent raw materials for cosmetic, nutraceutical, and pharmaceutical formulations [1, 2].
Although red raspberry (*Rubus idaeus)* is highly appreciated for fresh consumption, due its perishability, much of the fruit production is destined to industrial processing. Seeds, the main waste from this processing, are currently used to obtain a very appreciated cosmetic oil. However, cold pressing of raspberry seeds also produces an insoluble residue (Waste Raspberry Seed Powder, WRSP) that having not yet found any application represents an agro-industrial waste.
In this work, an ethanolic extract from WRSP was analyzed for its phytochemical profile. Moreover, functional value, including antioxidant, anticancer, and antimicrobial activities, has been evaluated
Phytochemical analysis, by both HPLC-ESI-MS/MS and spectrophotometric methods, revealed a very high content of polyphenolic compounds, in particular, flavan-3-ols, flavonols, and proanthocyanidins. The extract possess very high radical-scavenging and metal-reducing activity and it is able to prevent, at very small concentration, the lipid oxidation in a cell-based model. The WRSP extract also exhibited antiproliferative activity against three different epithelial cancer cell lines (MCF-7, HepG2, and HeLa cells) in a dose-dependent manner. Finally, microbiological assays showed a large inhibition spectrum against spoilage and pathogenic bacteria, without inhibitory activity against pro-technological bacteria.
The obtained results demonstrated that the waste generated by the cold pressing of red raspberry seeds could be a raw material for the extraction of bioactive compounds finding potential applications in the nutritional, nutraceutical, and pharmacological fields.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Identification of new small molecules for the treatment of lymphoma

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Lymphomas are blood malignancies generated from lymphocytes, recurring among the ten most common cancers in developed countries. Although some progresses have been achieved along the years in increasing survival, many patients still succumb because of this disease. Hence, more efficacious approaches and better therapeutic options for refractory forms are needed. Small molecules based on heterocyclic scaffolds constitute an important class of natural and synthetic products, widely used in the treatment of lymphoma in combination chemotherapy regimens. The G-protein-coupled receptors (GPCRs) play a key role in cellular physiology and homeostasis, and disruption of their pathways is associated with various diseases such as cancer. In particular overexpression, deletion or mutation of GPCRs are associated with the development of different types of Non-Hodgkin's lymphomas (NHL). My research group has already synthesize tricyclic [1,2]oxazole-based compounds, among which [1,2]oxazolo[5,4-*e*]isoindoles **1** and pyrrolo[2',3':3,4]cyclohepta[1,2-*d*][1,2]oxazoles **2** showed not only relevant results against the NCI 60 human tumor cell line panel, but also potent growth inhibitory effect against four different lymphoma histotypes with GI_{50} values in the low micromolar-nanomolar range.[1] The most sensitive cell line was found to be GCB-DLBCL (SUDHL10) expressing the GPCR olfactory receptor OR13A1 (ORs). On the basis of this results with the aim to identify new potent small molecules for the treatment of lymphoma, we planned to evaluate new structural modifications of the tricyclic core to obtain optimal candidates targeting GPCRs.

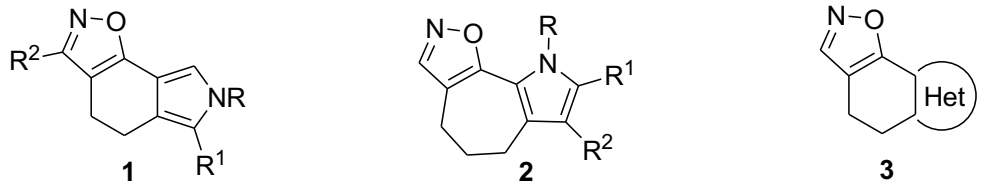

*Figure. [1,2]oxazolo[5,4-e]isoindoles **1**, pyrrolo[2',3':3,4]cyclohepta[1,2-d][1,2]oxazoles **2**, tricyclic [1,2]oxazole-based compounds **3**.*

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Influence of CX3CL1 on Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia affecting nearly 45 million people worldwide. The main neuropathological features of AD are the accumulations of β-amyloid plaques, tau tangles, neuroinflammation, and synaptic and neuronal loss, the latter being the strongest correlating factor with memory and cognitive impairment in AD [1]. The cerebrospinal fluid (CFS) obtained from AD and not AD patients were analyzed for the presence of the soluble form of CX3CL1, together with the canonical markers by ELISA test. The results obtained highlighted the increase of expression of CX3CL1 in subjects with AD compared to non-AD subjects [2]. Through an *in vitro* study on a neuron-astrocyte-microglia co-culture system, we have analyzed the effects of this cerebrospinal fluid (CSF) samples. Morphologically, treatment with CSF from AD patients showed a loss of neurofilaments and spheroids. Suggesting the presence in the CSF of elements destabilizing the neurofilaments, cellular adhesion processes, and intercellular contacts. Immunofluorescence assays showed an increased expression of p38 and fraktaline by AD CSF compared to the non-AD patients and not treated co-cultures. By Zymography, the expression of proteolytic enzymes was valued in cell extracts and the co-cultures conditioned medium; results indicated MMPs cascade activation by elements present only in the CSF obtained by AD patients. In Q-PCR assays, the expression of the inflammatory transcription factor, MMPs, and other Alzheimer's-related factors, show exciting differences.

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The role of Plant Growth Promoting (PGP) bacteria in the growth of Mediterranean diet plants producing high-value compounds

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Loss of fertility, together with drought and aridity, results in soil unproductivity and eventually desertification. Unsustainable agricultural practices are currently stressing the soil: an undervalued resource that, in addition to food, provides a multitude of invaluable services such as regulating the microclimate, reducing contaminants, and capturing CO_2. Hence, there is an increasing need to resort to sustainable approaches toward agricultural systems. Although Sicily is one of those regions most affected by water shortages, it is at the same time a reservoir of drought-resistant Mediterranean plants with high nutritional value and a rich microbiota. This project aims to promote agriculture based on Plant Growth Promoting Rhizobacteria (PGPR) [1] in order to reduce soil stresses. These microbes exert beneficial effects on plants that can be classified into direct such as nutrient solubilization and production of plant hormones (auxins, cytokinins, gibberellins), and indirect such as protection against pathogenic microorganisms [2]. Therefore, in the not-too-distant future, they could be used on a large scale in agricultural soils. Beyond the cited growth-promoting activity, is still unknown their role in increasing the production of bioactive molecules in plants: some of the best-known belong to the class of flavonoids, pigments, and terpenoids [3]. Within this context, benefits obtained from the interaction of these bioactive molecules would enhance a multitude of both physiological and epigenomic effects on the human body and would be provided by all-natural resources [4]. In a context where the concern for human health is primary over all other needs, the use of fruit extracts or concentrated oils rich in bioactive substances would contribute to an improved quality of life, using these products in both nutritional and agricultural fields. The innovative potential of using microorganisms in the agri-food system would lead to the mitigation of the use of plant stimulants, antibiotics, and chemical pesticides. Therefore, a focus will be made on the rhizosphere of all those plants capable of returning a high content of these compounds.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Functionalization of halloysite with protoporphyrin IX as potential system for photodynamic therapy

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The porphyrins are an important class of naturally fluorescent occurring macrocyclic compounds found in biological compounds that play a very important role in the metabolism of living organisms. From a biological point of view, porphyrins can be used as photosensitizers for photodynamic therapy (PDT). However, porphyrins show several disadvantages, such as low water solubility, cutaneous photosensitivity, and reduced selectivity for targeted tissues which hampered their clinical use.

Halloysite is an aluminosilicate clay belonging to the kaolin group with a typical hollow tubular structure and dimensions in the nanometric range (HNTs). Halloysite nanotubes are biocompatible nanomaterials, available in large amounts at low cost, capable to penetrate the cellular membranes, focalized themselves in the cytoplasm. In the last years, the modification of halloysite with different biological active species allowed to synthetize valuable carrier and delivery systems.[1]

Herein we report preliminary studies about the covalent modification of halloysite nanotubes with protoporphyrin IX to develop potential systems for PDT. To do this, two different experimental strategies were adopted, both traditional and innovative. The obtained nanomaterials were characterized by FT-IR spectroscopy, thermogravimetric analyses, dynamic light scattering and ζ-potential measurements and XPS analysis. The morphology was investigated by transmission electron microscopy (TEM) as well. Furthermore, the photoluminescent properties of the nanomaterials were investigated both in solution and in solid state.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
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Marine macroalgae: biodiversity and applications

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The research activity deals with several aspects of biology, systematic and ecology of marine algae, with a special focus on macroalgae inhabiting the Mediterranean Sea. In particular, the research focus on: 1) Alien marine macrophytes (e.g. *Caulerpa*, *Asparagopsis*, *Halophila*, Figure 1) and their interactions with autoctonous macrophytes, with particular attention to Marine Protected Areas [1]. 2) Coralline red algae (Rhodolits, *Lithophyllum byssoides*), characterized by a carbonate thallus, which form complex structures that increase the habitat biodiversity, and are a key component in the cycle of carbonate budget. Thus, they are of high interest for conservation [2]. 3) Brown algae belonging to the genus *Cystoseira sensu latu*, considered important habitat formers in the Mediterranean Sea, forming communities of great ecological value but at the same time highly threatened [3]. 4) Inter and intraspecific variations of secondary metabolites produced by brown algae as chemical defences and their biological application [4]. 5) Citizen science activities on the monitoring of alien marine macrophytes [5].

Figure 1. Asparagopsis taxiformis and *Caulerpa cylindracea* (photos by P. Balistreri).

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Be careful on DNA methylation alterations

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DNA methylation is an epigenetic modification involved in DNA compaction and in the regulation of gene expression. Also, DNA methylation is found in repetitive DNA including centromere and telomere. Recently, the research has been focused on repetitive sequences at the centromere due to its role in chromosome segregation, an event essential to cell fitness.
So far, studies revealed that tumour cells are usually hypomethylated at repetitive sequences and that DNA hypomethylation leads to the loss of chromatid cohesion and to aneuploidy as well[1-2]. However, in the centromeric sequences there are no coding genes so it is still unclear what role DNA methylation plays in this context. In order to answer this question we used and compared immortalized (RPE-1) and tumour (DLD-1) cell lines, both engineered to allow an inducible DNA hypomethylation (in collaboration with Institut Curis, Paris). We took advantage of the Auxin Inducible Degron (AID) system[3] to degrade the endogenous AID-tagged DNMT1 (DNA-methyl-Transferase1). The induced DNMT1-AID degradation for several cell cycles leads to passive DNA hypomethylation.
Our results showed that, once hypomethylated, immortalized non-tumour cells suffered a reduced growth rate. Both cell lines acquired aneuploidy and chromatids cohesion defects. We also observed an increase of mitotic errors, especially misaligned and lagging chromosomes, which strongly suggest a centromere/kinetochore malfunctioning. To this regard, by microscopy we noticed a reduced amount of centromeric proteins (CENPs) at centromere. Lastly, cells underwent nuclear and cytoskeleton defects that could also contribute to the genetic instability typical of tumour cells.
In summary, our study reveals that methylation of repetitive DNA is indeed important to cells survival and fitness, probably by maintaining centromere stability and function and by impacting on nuclear/cytoskeleton mechanics. This also suggests cautiousness in the use of hypomethylating drug as anti-cancer therapy that may have a risky effect on normal cells.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
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Organic Salts and non-conventional solvents: from organized reaction media to self-assembled functional materials

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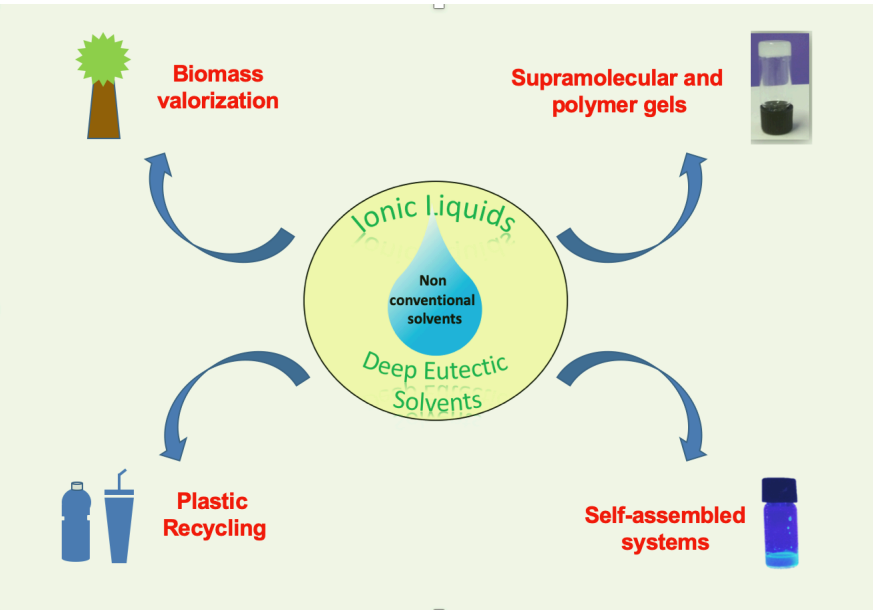
The research lines pursued in the group of Prof. Francesca D'Anna stems from the properties of non-conventional solvents like Ionic Liquids (ILs)[1] and Deep eutectic Solvents (DES).[2] Underpinned by non-covalent interactions, these solvents can be considered as supramolecular fluids. Moreover, their properties can be easily tailored to fulfill a given function or to enhance their sustainability, in terms of low eco- and cytotoxicity and components deriving from renewable resources. In this contribution we will describe their application in biomass valorization, supramolecular and polymer gels for environmental remediation, confined reaction media and materials with biological and antimicrobial activity, as well as plastic chemical recycling and self-assembled materials.



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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Comparison of polymeric networks based on POSS as catalytic bifunctional platforms for the conversion of CO_2 with epoxides.

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One of the most relevant pathways to transform CO_2 into valuable chemicals, is represented by its reaction with epoxides to give the corresponding cyclic carbonates. [1] This is a process that highly satisfies the principles of green chemistry and also, it is well known that materials bearing both nucleophilic and Lewis acidic species simultaneously can catalyze the process under mild conditions. [2-3] In this context, four heterogeneous bifunctional catalysts were prepared. The synthetic procedure involves a radical copolymerization of an octavinylsilsesquioxane as inorganic core building block and a Zinc or Aluminium porphyrin (**TSP-Zn** or **TSP-Al**) in presence of an imidazolium salt bearing a specific counter anion (chloride, bromide or iodide). The obtained solids **POSS-Zn-Cl**, **POSS-Al-Cl**, **POSS-Al-Br** and **POSS-Al-I** were used to investigate the co-catalytic effect of two different metals (Al and Zn) and also, the influence in the catalytic activity of different nucleophilic species (Cl^-, Br^- and I^-). All the catalysts were fully characterized and tested in the synthesis of cyclic carbonates starting from CO_2 and styrene oxide. Among all, **POSS-Zn-Cl** had the worst catalytic activity showing thus the better performance of aluminium as Lewis acid center. Moreover, **POSS-Al-Cl** was the less active in comparison to **POSS-Al-Br** and **POSS-Al-I**, but the latter two materials were not recoverable. On the other hand, **POSS-Al-Cl** was easily recoverable and used in four consecutive runs in the reaction with epichlorohydrin and carbon dioxide.

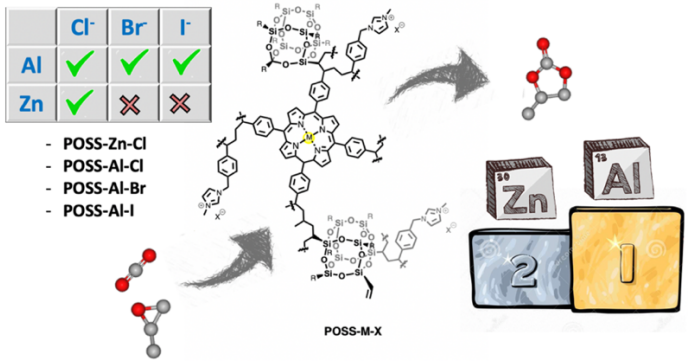

***Figure**. Schematic presentation of the work*

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Evaluation of Nutrigenomic Potential of Natural Stilbenoids

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Among natural phenolic compounds, stilbenoids are largely found in several fruits and crops, including blueberries, cranberries, peanuts and grapes. Stilbenoids are well known for their antioxidant, anti-inflammatory, anti-diabetic, anti-aging and anti-cancer properties, with trans-resveratrol and its dimethylated analogue trans-pterostilbene being the most deeply investigated compounds of this family [1]. Recently it was demonstrated the ability of trans-resveratrol, trans-pterostilbene and their dimers (±)-trans-δ-viniferin and (±)-pterostilbene-trans-dihydrodimer to target DNA [2].
The aim of the present work, performed in collaboration with IRIB-CNR of Palermo and DeFens Department of University of Milan, was to evaluate the biological effects of natural stilbenoids found in Vitis vinifera, with a focus on their activity as epigenetic modulators.
Differentiated Caco-2 cells as a model of the intestinal epithelial barrier, and HepG-2 cells 2 as a model of hepatic environment, were treated, with selected stilbenoids as resveratrol, pterostilbene and their dimers (±)-trans-δ-viniferin, (±)-trans-pterostilbene dehydrodimer. Furthermore 5-azacytidine (5-azaC) and Bobcat339-chlorhydrate (B339C) were used as reference compounds of DNA methylation. Stilbenoids were evalued for their toxicity, ability to interact with DNA, and epigenomic action. Activity on DNMTs and TET enzymes was evaluated as well as genome-wide DNA methylation changes. Resveratrol, pterostilbene, and (±)-trans-pterostilbene dehydrodimer were found to have no toxic effects at tested concentration chosen to mimic actual concentrations in gut lumen and liver [14]. Furthermore, the analysis of genomic DNA methylation showed a significant demethylation of genomic DNA in both cell lines, when treated with (±)-trans-δ-viniferin, 5-azaC and B339C. Moreover, it was assessed the recovery property of selected stilbenoids after an (epi)mutagenic pre-treatment with arsenic, a natural pollutant contained in drinking water. Both resveratrol, pterostilbene, and (±)-trans-pterostilbene dehydrodimer were effective in reversing arsenic damage in differentiated Caco-2 cell lines. (±)-trans-δ-Viniferin showed epigenomic activity, but further studies are needed to clarify its mode of action.

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New insights of epithelial-to-mesenchymal transition (EMT) signature in breast cancer

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The epithelial-to-mesenchymal transition (EMT) is a complex, stepwise process that occurs during embryonic development and tumor progression. The loss of epithelial phenotypic characteristics and the acquisition of mesenchymal traits represent the hallmark of EMT. Accumulating evidence suggests that EMT program helps cancer cells to acquire invasive and metastatic behaviors and enhances cell survival [1,2]. The EMT is a fundamental process in the progression of breast cancer (BC), an exceptionally heterogeneous disease with high rate of intratumor heterogeneity, and patients diagnosed at the same stage of the disease show very different clinical responses and survival periods. In this study, bioinformatics and proteomic approaches were used to analyze the molecular and cellular mechanisms of the EMT in breast cancer, to outline an EMT-signature, useful for patients' stratification.A subset of specific EMT mediators in breast cancer was extracted by EMTome database [3] and further analyzed by UALCAN and Kaplan-Meier Plotter databases. A list of 49 genes was selected because differentially expressed between normal and breast cancer tissues and significantly associated with prognosis. The obtained list was analyzed by using the FunRich database to verify the functional enrichments of selected EMT-genes. Interestingly, the selected EMT mediators in BC were found functionally associated with syndecan-2 and enriched within the exosomes, pointing to the importance of the microenvironment. Proteomic expression of vimentin and E-cadherin, two important markers of the mesenchymal and epithelial phenotypes, was verified by western blotting on 99 breast cancer tissues. A high heterogeneity of expression of both E-cadherin and vimentin was found in the cohort of analyzed patients. Surprisingly, no correlation between the expression levels of vimentin and E-cadherin was found, suggesting that the EMT in breast cancer could be more complex than previously assumed. Furthermore, we demonstrated the existence of different forms of vimentin with different molecular weights, whose functional significance deserves further investigations. In conclusion, a combination of bioinformatics and proteomic data revealed a possible EMT-related gene signature in BC, biologically interconnected, and demonstrated in BC tissues a remarkable heterogeneity of expression of vimentin and E-cadherin. Further studies on the connection between EMT markers and BC will contribute to identify the clinical significance of this heterogeneity.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Bacterial biofilms for environmental bioremediation

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Bioremediation is a promising technology for the treatment of polluted environments based on the biodegradation capacities of native or introduced microbial populations. Bioremediation is traditionally carried out using free bacterial cells, though utilization of immobilized bacterial cells on adsorbing matrices is a promising technique due to biotechnological and economic benefits. Bacterial biofilms show greater resilience, survival and degradative activity for longer periods than cells in the planktonic state. A bioremediation system was developed immobilizing highly performant hydrocarbon (HC)-degrading bacteria on biodegradable oil-absorbing biopolimeric carriers. Soil HC degrading Actinobacteria *Nocardia cyriacigeorgica* SoB, *Gordonia amicalis* SoCg [1], and marine hydrocarbonoclastic Gammaproteobacteria *Alcanivorax borkumensis* AU3-AA-7 [2] were immobilized on polylactic acid (PLA) and polycaprolactone (PCL) membranes prepared by electrospinning [3]. The capacity of adhesion and proliferation of bacterial cells into the biopolymers were evaluated using scanning electron microscopy (SEM) after 5, 10 and 15 days, and their survival was monitored over time simulating storage effects. PLA and PCL nanofibers were covered by bacterial cells already after 5 days incubation. Total biomass (estimated as total dsDNA) extracted from biofilms confirmed the colonization up to 15 days incubation. Viable plate counts showed that survival of the bacterial strains was high for the entire experimental period, and bacterial biofilms adsorbed on biopolymers were still viable after 30 days. HC biodegradation ability of biofilms, assessed by GC-FID analysis, resulted higher in respect to the corresponding free-living bacterial cultures. Expression of the biodegradative genes in biofilms are in progress.

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Innate or acquired resistance to anticancer drugs: our experience to overcome it

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Generally, the pharmacological treatment of tumors takes place through polytherapy to lower the doses of individual chemotherapy and prevents as much as possible the selection of cells resistant to the drugs administered. In the last decade, targeted cancer therapies have been developed and entered clinic in the hope of improving anticancer efficacy. While many of the targeted therapy drugs showed promising early clinical outcomes with improved overall survival, many patients receiving targeted therapy developed drug resistance. Drug resistance, which is often of a multiple type, can be defined as the ability of cancer cells to obtain resistance to both conventional and novel chemotherapy agents, and remains a major problem to resolve in cancer therapy. The mechanisms of resistance are multifactorial and, in our cellular models of acute myeloid leukemia, hepatocellular carcinoma and triple negative breast cancer, involve the NF-κB pathway. In the phenomena of both innate and acquired drug resistance more strictly pharmacological factors are certainly involved, such as the overexpression of multidrug efflux transporters belonging to the superfamily of the ATP-Binding Cassette type proteins (ABC) P-glycoprotein, and biological factors such as inhibitory of apoptosis proteins (IAPs), Raf-1 kinase inhibitor protein (RKIP), an important tumor suppressor and metastasis inhibitor, which enhances drug-induced apoptosis of cancer cells and Yin Yang 1 (YY1) a transcription factor involved in drug resistance [1].
It is also relevant to consider the role that variations in the tumor microenvironment can play in establishing a resistant phenotype. A recent article highlights how microvesicles (MV), deriving from resistant cell lines that are freed directly from the plasma membrane, can transport and transfer P-gp and IAPs molecules to sensitive cancer cells, which can then establish drug resistance and that this phenomenon is related to the increase in NF-κB expression [2]. Natural compounds have been shown to interfere with MDR P-gp mediated with some pharmacokinetic limitations. To improve the solubility and bioavailability of these substances, we are studying the possibility to convey the efflux pump inhibitors in nano-formulations to tumor cells that overexpress them [3].
In our opinion, multitarget molecules can be considered as privileged compounds capable of attacking and reversing the resistant phenotype. In this context natural compounds can represent a valid multitarget strategy to combat drug resistance.

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Blue light-mediated photocatalysis for antibacterial photodynamic therapy (aPDT)

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When exposed to light, titanium dioxide (TiO_2) produces a cascade of radicals that can induce photodegradation in various targets such as biomolecules, bacteria, or biofilms: that's why TiO_2-based materials are of great interest for biomedical applications. In bare materials, these reactions are triggered by UV light, which, as is well known, may induce toxic side effects and has limited penetration into tissues[1]. To overcome these limitations, synthetic methods with nitrogen doping have been used to modify the bandgap of the semiconductor[2]. We here present an experimental study by means of spectroscopy and microscopy methods aimed at the production, and characterization of nitrogen-doped TiO_2 (N-TiO_2) nanostructures and analyzing their photocatalytic activity in combination with gold nanoparticles (AuNPs) as suitable enhancers with well-known biocidal and tunable optical properties. N-TiO_2 nanostructures were shown to readily induce photodegradation of the model dye Methyl-orange in solution, under illumination with blue radiation (at 420 nm). Interestingly, the photocatalytic activity of these structures was demonstrated to be enhanced in the presence of AuNPs. A possible explanation of this result is that the inclusion of gold on the nanostructured surface of N-TiO_2 enhances the separation of photogenerated electron-hole pairs and increases interfacial charge transfer[3]. Studies are currently in progress to assess the photodegradation of biomolecules such as proteins and DNA, while preliminary studies on bacterial cultures demonstrate antibacterial activity following LED irradiation, making them suitable as photo agents for antibacterial photodynamic therapy (aPDT). More research is needed to properly study these processes in composite materials in which titanium and gold are included in hydrogels, nanofibers, or films with selected components to improve biocidal efficacy.

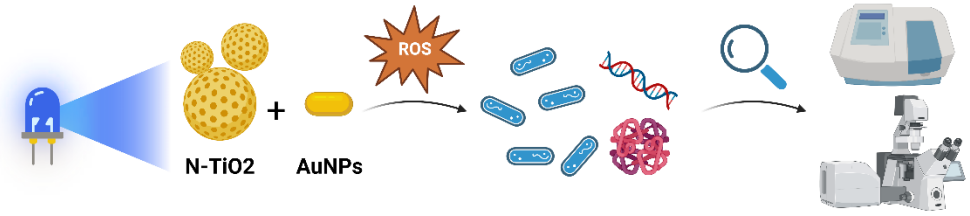

Figure: Graphical abstract

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From sea urchin to human and from molecular biology to functional genomics

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Deuterostomes include the phyla Echinodermata and Chordata. For this reason, seemingly different animals like sea urchin and human are actually very close, not only regarding mechanisms of development, but also molecular pathways and nucleic acid sequences.
The research interest and expertise in our laboratory has been evolving in the last years, like the evolutionary path from echinoderms to humans. Starting from the analysis of gene structure and gene expression of small multigenic families (such as tubulins and metallothioneins in sea urchin), our interest now lies on large multigenic families (such as the Toll-like receptor one in sea urchin, composed of 120 members [collaboration with S. Costa, Dept. STEBICEF, and A. Nicosia and R. Russo, CNR]) and DNA methylation dynamics in human cells.
In particular, we performed meta-analyses of genome-wide DNA methylation and integrative OMICs, looking for biomarkers of HPV infection and cervical cancer progression, and reanalyzing previously published methylome datasets (from methylation profiling by array and Whole-Genome Bisulfite Sequencing experiments) [collaboration with G. Capra, Dept. PROSAMI].
Currently, we are analysing new Reduced Representation Bisulfite Sequencing (RRBS) datasets [collaboration with F. Caradonna and C. Gentile, Dept. STEBICEF], using different ***R*** *packages*, with the following aims: investigating the DNA methylation landscape in CaCo-2 cells, detecting differentially methylated regions (DMRs) after their exposure to indicaxanthin (a type of betaxanthin present in prickly pears cactus), characterizing the genomic features that are associated with DMRs, such as CpG island, DNAseI hypersensitive regions, histone modifications, promoter/enhancer annotations.

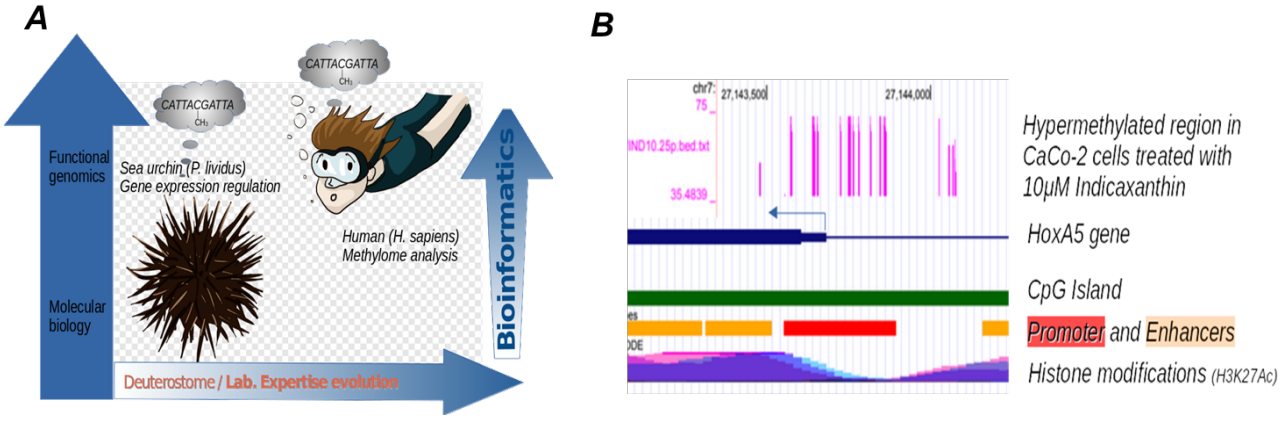

Figure. A: Cartoon representation of our lab. expertise development during the last years. B: RRBS results visualized in the UCSC genome browser.

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Synergic effect of ultrasounds and ionic liquids in the recycling of polycarbonate

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In the last decades, the use of plastic is continously increasing, because it is an inexpensive and adptable material. On the other way, the incenssant use of plastic led to worrying envionmental pollution. The growing concern about plastic pollution has stimulated the investigation of methodologies for plastic recycling. In this context, we investigated the depolymerization of polycarbonate into bisphenol A. In this regard, we combined basic task specif ionic liquids (TSIL) and ultrasounds to carry out the depolymerization of polycarbonate (PC). In particular, we used TSIL as catalysts, and methanol as solvent. We also performed propanolysis, butanolysis and glicolysis of polymer, as well as depolymerization of PC waste deriving from DVD or PC- based sheet.

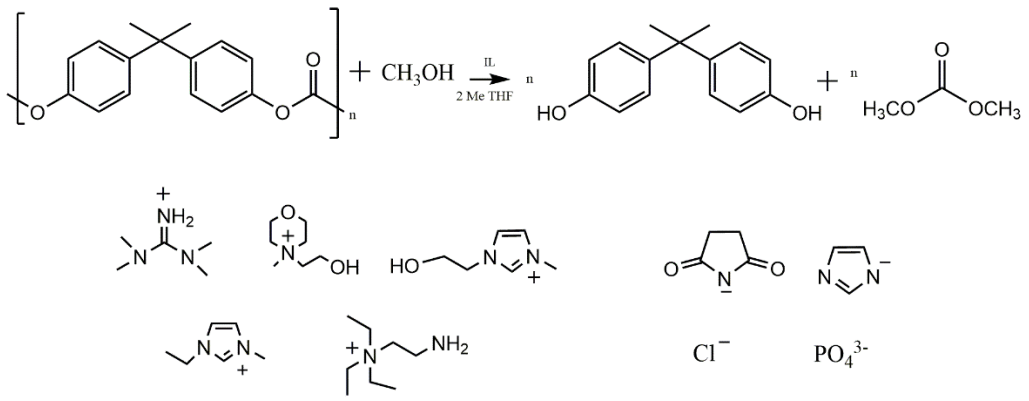

Figure 1: Methanolysis reaction ; cation and anion of IL

The results showed that the combination of TSIL and ultrasound is an efficient strategy to carry out the depolymerization of polycarbonate (PC), allowing the reaction to be performed under mild conditions, at 30°C, in agreement with the principles of Green Chemistry.[1] Furthemore, cytotoxicity tests conducted on the best performing catalysts, revealed of their safe character.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Actinomycetes: a still-to-tap source of bioactive secondary metabolites

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According to the World Health Organization, antibiotic resistance, namely the ability of bacteria to survive and replicate in the presence of an antimicrobial drug, is today one of the biggest threats to global health. Thus, the challenge of the pharmaceutical industry is to develop novel antibiotics. Secondary metabolites are low molecular weight molecules with complex chemical structures and biological activities, such as antibiotics, antitumor agents, immunosuppressive agents, inhibitors, and enzymes. Actinomycetes are the most economically and biologically precious bacteria for producing secondary metabolites of industrial interest.
Actinomycetes are Gram-positive bacteria characterized by a complex morphological and physiological differentiation, featuring genomes with a high GC content, and commonly found in soil. We aim to search for new actinomycetes in unexplored niches (i.e., hydrothermal vents, activated sludge, and cultural artefacts) to discover new bioactive compounds.
So far, our collection consists of hundreds of actinomycetes. 108 soil isolates were evaluated for the production of novel antibiotics against Gram-positive and negative bacteria with a multi-resistance profile. Ten potential antibiotic producers were collected and used for metabolomic analysis to evaluate the production of secondary metabolites. In addition, novel cultivation strategies and epigenetic approaches have been undertaken to enhance the discovery of new secondary metabolites holding intriguing antimicrobial activities.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Computational studies for the identification of new Sirtuin-6 inhibitors in the treatment of lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL), accounting for about 22% of newly diagnosed cases of B-cell NHL worldwide with more than 18,000 new diagnoses each year(1). Sirt-6 is a highly conserved ADP-ribosylase and NAD^+-dependent deacylase involved in broad cellular processes. It is closely related to DLBCL and strictly associated to poor prognosis due to drug resistance occurrence and tumorigenesis mediated by PI3K/Akt/m-TOR pathway(2). Considering the fast development of the computational chemistry and biology, the aim of the project is the rational design of new Sirt-6 inhibitors supported by the artificial intelligence. Molecular docking, molecular dynamics, and molecular metadynamics studies are used to evaluate the pharmacophore features required to bind the protein active site, thus providing new information for the rational design of potential new Sirt-6 inhibitors. A virtual screening campaign was performed on a commercial database and *in-house* library leading to the identification of 32 hits that will be synthesized and submitted to biological assessment.

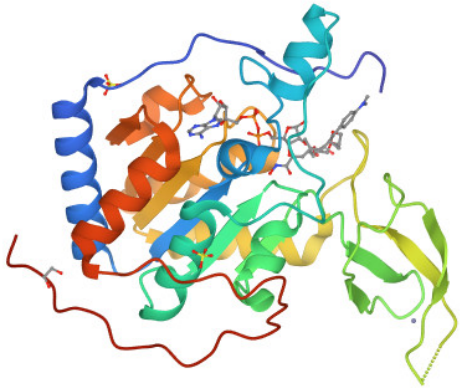

Figure 1. *Human Sirt-6 in complex with ADP-ribose and the inhibitor Trichostatin A (PDB: 6HOY).*

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Biochemical and molecular bases of mammalian nervous system development and maturation

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Our Research Group is involved with projects aimed at studying the biochemical and molecular bases of mammalian nervous system development and maturation. A few examples are given below:

1. In order to understand the mechanisms underlying formation and maintenance of the Blood-Brain barrier (BBB), and to analyze the capability of specific drugs and pro-drugs to cross BBB, we set up a three cell types *in vitro* model of BBB (endothelial cells, neurons and astrocytes), that allowed us to analyze the effects of neurons on differentiation of brain capillary endothelial cells (RBE4.B cells) in culture.
2. We demonstrated that all the cell types of the Nervous System are able to release of extracellular vesicles (EVs); for example, both neurons and astrocytes shed EVs containing FGF-2 and VEGF. On the other hand, oligodendroglioma cells (OCs), like other malignant cells, release a much higher amount of EVs, which contain tumour-specific proteins, and RNAs. EVs from OCs also contain pro-apoptotic factors, that can induce apoptosis in both rat cortical neurons, and astrocyte. In order to study vesicle release in a system that can better resemble *in vivo* conditions, astrocytes and BCECs were also cultured on poly-L-lactic acid (PLLA) scaffolds to which they adapted well, also showing a high capacity to release EVs (Carfì Pavia 2019, Di Bella 2017).
3. We have been also studying since long ago regulation of the expression of the histone linker variant H1.0, for which we showed that synthesis is mainly regulated at post-transcriptional level, and depends on RNA-binding proteins (RBPs). We previously identified RBPs apparently specific for this messenger and, in particular, cloned two novel proteins by screening an expression cDNA library by binding to radiolabeled RNA: CSD-C2, also know as PIPPin, and PEP19.
4. Finally, in order to gain a better knowledge of the cellular and molecular events that characterize malignant cells, we purified, from cultures of rat primary astrocytes, some clones with increasingly high cell division rates. In collaboration with Prof. Caradonna we performed analysis of all these cells, at both cytogenomic and epigenetic level, that showed that the most modified astrocytes (A-FC6 clone) have epigenetic and chromosomal alterations typical of cancer, such as an isochromosome (i8q). We also performed, in collaboration with Prof.ssa Cancemi and Dott. Scilabra, proteomic analysis that allowed us to identify, in the EVs released from the A-FC6 clone, proteins, such as MMP3 metalloproteinase, that can modify the extracellular matrix thus allowing invasion.

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Coexistence of anaerobic and aerobic dechlorinating communities in 1,2-dichloroethane contaminated groundwater

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Chlorinated Aliphatic Hydrocarbons (CAHs) are widespread, persistent and toxic environmental pollutants. Bioremediation of CAHs-contaminated aquifers is generally achieved through anaerobic processes mediated by specialized microorganisms known as OrganoHalide Respiring Bacteria (OHRB)[1], but aerobic and co-metabolic processes can also co-occur[2]. The aim of this work is to investigate the intrinsic biodegradation potential of the autochthonous microbial communities from an aquifer mainly contaminated by 1,2-dichloroethane (1,2-DCA), in response to anaerobic and aerobic biostimulation treatments in microcosm. Microcosms under aerobic and anaerobic conditions were set up from contaminated groundwater using mineral salt media amended with 1,2-DCA and appropriate substrates, if required (lactate or no additive amendment in anaerobic microcosms; volatile hydrocarbon mixture or no additive amendment in aerobic microcosms). The bacterial communities from the aquifer and microcosms were characterized by 16S rRNA gene Metagenomic Sequencing and 16S rRNA OHRB specific probes. Although low relative abundances of known dechlorinating anaerobic and aerobic bacteria were detected in the aquifer, the biostimulation treatments allowed the enrichment of both anaerobic and aerobic 1,2-DCA dechlorinating communities as revealed by the removal of 1,2-DCA monitored over time by GC-MS. Known dechlorinating genera detected in the anaerobic (e.g. *Dehalococcoides* and *Desulfuromonas)* and aerobic (*Starkeya* and *Ancylobacter*) enriched cultures may mediate the biodegradation of 1,2-DCA under anaerobic and aerobic conditions respectively. The presence of catabolic genes *rdhA* and *dhlA,* involved in reductive and hydrolytic dechlorination respectively, was confirmed by PCR assays with degenerated and specific primers and sequencing. In conclusion, although the site shows a poor intrinsic catabolic potential, the detection of aerobic and anaerobic 1,2-DCA degraders and dechlorination genes in enriched cultures suggests the coexistence of aerobic and anaerobic metabolisms that could be biostimulated and exploited for the bioremediation of the site.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

RNA Editing Approaches for the correction of nonsense mutation in a cell model for Cystic Fibrosis

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Cystic Fibrosis (CF) is an autosomal recessive disease caused by mutations in the *CFTR* gene. In particular, *CFTR* nonsense (STOP) mutations generate a premature termination codon (PTC) in the mRNA, leading to the production of a shortened and non-functional protein[1]. Currently, there is no pharmacological therapy that specifically targets nonsense mutations in CF. In this regard, we are exploring the possibility to correct the PTC using different RNA-based editing tools. These systems exploit the Adenosine Deaminases Acting on RNA (ADAR) to convert the adenosine within the PTC into inosine and allow the full-length protein synthesis[2]. Among these, a compact REPAIRv2 system uses a modified and truncated dCAS13x.1 fused with $ADAR2_{DD}$ that is recruited to the adenosine of PTC by means of a specifically designed guide RNA[1]. A different system named RESTORE uses specific antisense RNA oligonucleotides (ASOs), complementary to the CFTR mRNA region with the PTC, except for a cytidine-adenosine mismatch that promotes ADAR recruitment[3]. In addition, we also evaluated phenotypical anomalies of CFTR mutated cells showing morphological differences in comparison to wild type cells[4]. Our results pave the way to new therapeutical strategies potentially able to correct the nonsense mutations in cystic fibrosis.

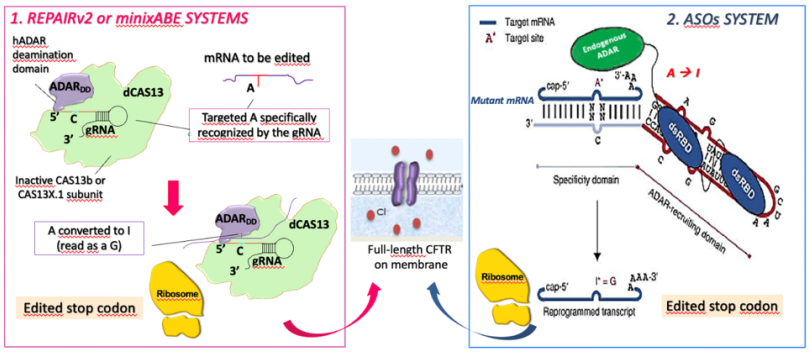

Figure 1. Schematic representation of RNA editing by CRISPR-dCAS13/$ADAR2_{DD}$ and ASOs systems.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
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Spray-Drying: a Versatile Technique for Producing Gastro-Resistant Microparticles

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In the present study, gastro-resistant microparticles (MPs) were produced using the spray-drying technique as controlled-release systems for some model liposoluble vitamins, including retinyl palmitate, retinyl-acetate, β-carotene, cholecalciferol, and α-tocopherol [1]. The gastroprotective action of three different gastro-resistant excipients, the anionic methacrylic copolymer (Eudraguard® Biotic, E1207), the cellulose acetate phthalate (CAP), and whey proteins (WPs), was compared [2] [3]. The latter was used to produce a novel delivery system manufactured with only food-derived components, such as milk, and showed several improvements over the two synthetic gastro-resistant agents [4] [5]. Scanning electron microscopy (SEM) images showed a quite homogeneous spherical shape of all microparticle batches, with an average diameter between 7 and 15 μm. FTIR analysis was used to evaluate the effective incorporation of vitamins within the microparticles and the absence of any degradation to the components of the formulation. The comparison graphs of differential scanning calorimetry (DSC) confirmed that the spray drying technique generates a solid in which the physical interactions between the excipients and the vitamins are very strong. Release studies showed a prominent pH-controlled release and partially a delayed-release profile. Ex vivo permeation studies of retinyl palmitate, retinyl acetate, and α-tocopherol revealed greater transmucosal permeation capacity for microparticles produced with the WPs and milk.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Design and synthesis of new 2-phenyl-ethenylquinolin-4-methanones as FGFR4 inhibitors

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The Fibroblast Growth Factor Receptor 4 (FGFR4) signalling pathway has been shown to be involved in several biological processes, including embryonic development, cell proliferation and differentiation. Its activation has also been closely associated with cancer development and progression in many human tumors with poor prognosis (hepatocellular carcinoma, colon cancer and malignant pleural mesothelioma). *In vitro* evidences proved that the inhibition of FGFR4 signalling is associated to apoptosis in tumor cells, thus suggesting FGFR4 might be a promising therapeutic target. Ponatinib, a clinically approved pan-FGFR inhibitor with nanomolar efficacy, is affected by several side effects due to its lack of selectivity and high lipophilicity.
Searching for new FGFR4 inhibitors, we performed computational studies leading to the identification of quinazolinone derivatives. Biological assays conducted at the Cancer Research of the Medical University of Vienna, demonstrated their ability to inhibit selectively FGFR4, thus deserving further investigation.
Since quinoline core perfectly overlaps with the 2-imidazopyridazine ring of Ponatinib, a new series of quinoline derivatives was designed and, based on docking scores and binding poses, 41 compounds were synthesized with the MAOS technique. Preliminary *in vitro* biological assays were conducted on NCIH1703 cell line at the Cancer Research of Vienna, showing promising IC_{50} values.

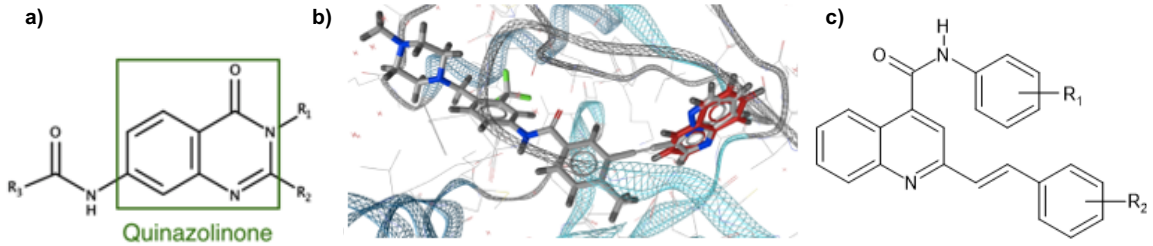

Figure 1. *a) Chemical structure of the quinazolinone derivatives; b) overlap of the quinoline core and 2-imidazopyridazine nucleus of Ponatinib; c) chemical structure of the new quinoline derivatives.*

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Zymographic Analysis of Matrix Metalloproteinases (MMP-2 and MMP-9) in Cerebrospinal Fluid and Sera from Patients with Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS), of supposed autoimmune origin, associated with neuroinflammation, demyelination, and neuroaxonal degeneration [1]. In MS the migration of immunocompetent cells into the CNS requires the opening of the blood-brain barrier (BBB), due to the extracellular matrix (ECM) remodelling [2]. The BBB breakdown is probably mediated by matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases classified according to their substrate affinities into collagenases, gelatinases, stromelysins, matrilysins, and membrane-bound/associated [3]. Among the MMPs, the gelatinases MMP-2 and MMP-9 play a central role in the immunopathogenesis of MS, through the disruption of the BBB and the recruitment of inflammatory cells into the CNS. The MMPs and their physiological inhibitors TIMPs are expressed in various types of cells including neurons, astrocytes, oligodendrocytes, and microglia. Previously, MMP-2 and MMP-9 have been proposed as candidate biomarkers for MS progression, and higher levels of MMP-2 and MMP-9 in serum and CSF of MS patients have been also reported [4]. Gelatin zymography is a simple and advantageous technique, useful to identify the enzymatic activity of both pro and active forms of MMP-2 and MMP-9 enzymes, as well as complexes of proMMP-9 with TIMP-1 or dimers of proMMP-9 in different biological fluids [5, 6]. In this study, we analysed the gelatinase activity levels in the cerebrospinal fluids (CSF) and serum samples of patients diagnosed with MS or other neurological disorders, considered as neurological controls (NC). The analysis included 90 subjects (59 patients with MS and 31 NC). Of both groups, a significant number of CSF (76) and serum samples (44) were analysed. Moreover, both CSF and serum of 22 patients were available. Our results confirmed a different expression of MMPs in CSF and serum. In particular, the active forms of MMP-2 and -9 were detected only in CSF samples, while the proMMP-9 dimers were revealed only in serum samples. ProMMP-9, proMMP-2 and proMMP-9/TIMP-1 were detected in both biological fluids. Interestingly, proMMP-9 levels were significantly higher in sera (p<0.001) while proMMP-2 levels were significantly higher in CSF (p<0.001). The Mann–Whitney U-test was applied to compare the expression levels of MMPs in both CSF and serum between MS and control groups. In CSF, the proMMP-9/TIMP-1 levels were higher in NC compared to RRMS, while proMMP-9 and MMP-9 levels were higher in RRMS compared to NC. A different trend was found for both proMMP-2 and MMP-2, with higher levels in NC compared to MS. Lastly, different levels of MMPs occurred during active phases of MS [i.e., clinical relapses and the presence of enhancing lesions on magnetic resonance imaging (MRI)]. An interesting aspect that deserves further investigation was to find an enrichment of MMP-2 in extracellular vesicles (EVs) isolated by CSF. In conclusion, this study confirms that MMP-2 and MMP-9 could be useful as potential biomarkers for monitoring MS disease activity and suggest that a shift in proMMP-9/TIMP-1 balance towards proteolytic activity of MMP-9 could be relevant in MS immune dysregulation.

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Bifunctional Mg-Porphyrin based Heterogeneous Catalysts for CO_2 Fixation

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The fixation of CO_2 into epoxides for the production of cyclic carbonates is a transformation (or a reaction) of considerable interest to the scientific community.[1] This is due to the possibility of transforming a waste into valuable chemicals.[2] Herein, as a continuation of our ongoing research,[3] highly cross-linked materials containing imidazolium salt and magnesium porphyrin in absence (**MgTSP-imi**) and in presence (**CNT-MgTSP-imi8** and **CNT-MgTSP-imi12**) of multi-walled carbon nanotubes (MWCNTs) were synthesized. The hybrid materials were prepared in a simple one-pot procedure and characterized by means of various spectroscopic and analytical techniques such as TGA, TEM, SEM-EDX, ICP-OES, XPS, solid-state NMR and N_2 physisorption. The solids were employed as heterogeneous bifunctional catalysts for the conversion of carbon dioxide and epoxides into cyclic carbonates. The results obtained were excellent in terms of conversion and TON_{Mg} values, highlighting the importance of using the co-catalyst species with Lewis acid functionality. Interestingly, **MgTSP-imi** exhibited better catalytic activity than the two systems supported on MWCNTS, but also compared to a reference homogeneous catalytic system, demonstrating that the close proximity between the metal centre and the nucleophilic site results in a synergistic effect during the catalytic cycle (Figure 1).

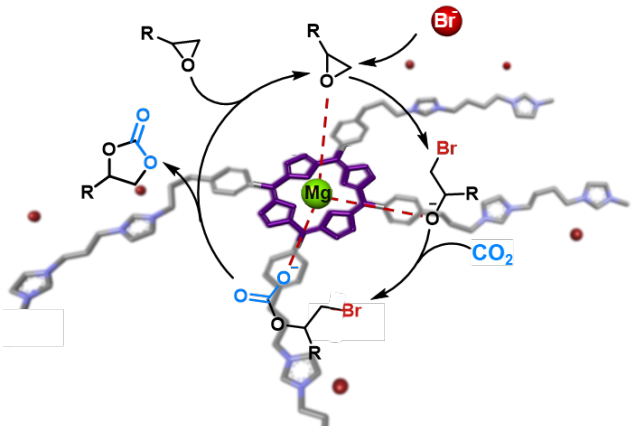

Figure 1: Synergy between the metal centre and the nucleophilic site in synthesis of cyclic carbonates.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

C_3N_4/Nb_2O_5 composites for photo-reforming in aqueous solution of oxygenated organic compounds

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Photocatalysis based on the use of semiconductors has gained, during the recent years, increased attention as promising sustainable process for H_2 and fine chemicals production, given the possibility of activating such photocatalysts by visible light radiation [1]. Photocatalytic treatments devoted to the conversion of organic substances contained in wastewaters into chemical compounds of high interest, such as hydrogen, represents a new strategy to recover chemical energy and an innovative route to decontaminate wastewaters. The photo-reforming of aqueous solutions containing oxygenated organic compounds combines photocatalytic water splitting with the oxidation of organic substrates in a single process occurring at ambient conditions. We have observed, by using TiO_2 as photocatalysts, that the generated H_2 comes both from water-splitting and from organic substrate dehydrogenation [2]. Unfortunately, the most active photocatalysts are active under UV irradiation, so further studies are necessary to improve the solar light absorption ability of the semiconductors. In this research, both bare and composite materials based on C_3N_4 and Nb_2O_5 have been used as photocatalysts for the photo-reforming of aqueous solutions of oxygenated compounds. The choice of C_3N_4 and Nb_2O_5 arises from the fact that their heterojunction can lead to lower recombination between the photo-generated electrons and holes. Furthermore, the potential of the conduction and valence bands of the two semiconductors are suitable for both the formation of hydrogen and the oxidation of the organics. Moreover, due to its favorable band gap, carbon nitride exhibit high visible light absorption. We are encouraged from the good results obtained by the C_3N_4/graphene heterojunction for triethanolamine photoreforming, obtaining an amount of H_2 under UV irradiation of ca. 88 $mmol \cdot g^{-1} \cdot h^{-1}$ with an apparent quantum yield of ca. 9 % [3].

In this work, Nb_2O_5 and Nb_2O_5/C_3N_4 composites have been prepared by hydrothermal synthesis. The photo-reforming activity has been measured under both UV-LED irradiation and natural sunlight illumination. Moreover, the presence of metallic Pt as co-catalysts has been studied.

The obtained results are encouraging, particularly under natural sunlight irradiation, because in some cases the hydrogen formation resulted higher than 40 $mmol \cdot g^{-1} \cdot h^{-1}$ with an apparent quantum yield of ca. 5 %.

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New 1,2,4-oxadiazoles derivates potentially active against SARS-CoV-2 main protease

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The COronaVIrus Disease 2019 (COVID-19), caused by the novel coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), continues to be a public health concern. Even though the interest of the scientific community in reducing its circulation, after a couple of years since the beginning of the pandemic, the therapies used have not been able to completely counteract the high number of infections.

The primary objective is to realize new drugs able to prevent the spread of the SARS-CoV-2 and to relieve the symptoms of the COVID-19, without suffering the virus' ability to mutate.

Therefore, the Main Protease (M^{pro} or $3CL^{pro}$) of the SARS-CoV-2 is considered an attractive target.[1] The M^{pro} is responsible for the cleavage of two polyprotein (pp1a, pp1ab) in 16 non-structural protein (Nsps) that form the replicase complex. Consequently, mutations on this enzyme are low, as they would be fatal for virus life. Specificity and high degree of conservation of proteases catalytic site in different coronavirus is used for new compound design. [2]

These new 1,2,4-oxadiazoles derivates (Fig. 1) could inhibit the catalytic activity of the SARS-CoV-2 M^{pro} in two different ways. Firstly, they could act irreversibly inhibiting the protease by mimicking its natural substrate (Fig.1 i), thanks to cysteine warheads that can covalently bind the catalytic site,[3] and subsequently reversing the dimerization process of the active enzymatic form (Fig. 1 ii). [4]

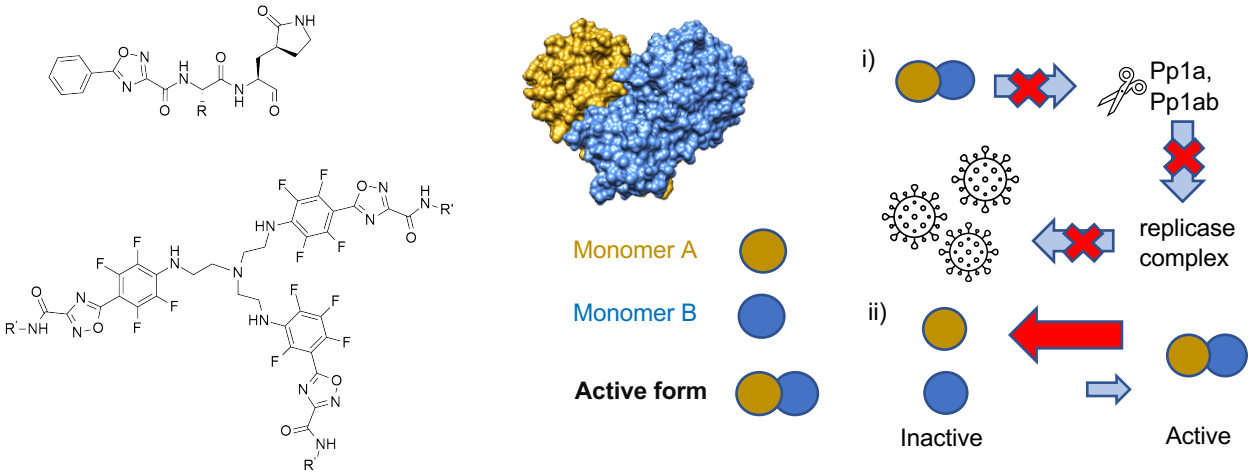

Figure 1. Structure of 1,2,4-oxadiazole derivates, schematic representation of M^{pro} and mechanism of inhibition.

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New SARS-CoV-2 M^{PRO} covalent inhibitors

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The viral Main PROtease (M^{PRO}) is one of the most attractive targets among all key enzymes involved in the SARS-CoV-2 life cycle. Inhibition of SARS-CoV-2 M^{PRO} with selective antiviral drugs halts the replication process of the virus without affecting the human catalytic pathways [1]. Covalent inhibition of the catalytic Cys^{145} of the SARS-CoV-2 M^{PRO} binding pocket (Figure 1, PDB code: 6Y2F [2]) has led to the development of covalent protease inhibitors [3]. In light of this, our research interest is focused on identifying new small molecules able to modulate SARS-CoV-2 M^{PRO} activity. Ligand/structure-based virtual screening, integrated with non-covalent and covalent docking protocols, allowed the identification of new compounds with optimal interaction with both the catalytic residue and the other clefts in the binding site. The in-silico selected molecules will be synthesized according to appropriate synthetic strategies and biologically evaluated with enzymatic inhibition assays and/or antiviral studies.

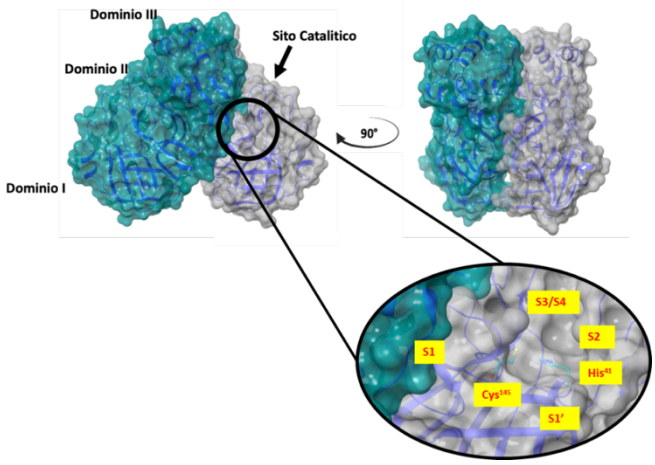

Figure 1. SARS-CoV-2 M^{PRO} X-Ray structure (PDB code: 6Y2F).

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE
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Beneficial impact of *Aphanizomenon flos aquae* (AFA) extract in countering obesity-related dysmetabolisms in HFD-obese mice.

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Aphanizomenon flos aquae (AFA) is a unicellular cyanobacterium spontaneously growing in Upper Klamath lake (Oregon, USA) and considered a superfood due to its health-enhancing properties [1]. AFA is an important source of the blue photosynthetic pigment phycocyanin, beta carotene and chlorophylls which have been described as natural substances with strong antioxidants and anti-inflammatory properties [2].
Several scientific evidence showed AFA's ability to counteract the progression of chronic disease [2]. However, there remains a paucity of data on the anti-obesity potentials of this microalgae.
In the present study we investigate the effect of KlamExtra (a commercialized AFA extract) supplementation on obesity-related metabolic disorders in a mouse model of high-fat diet (HFD)-induced obesity. To this end, biochemical and histological analysis, oxidative stress, and inflammation evaluations in the liver and adipose tissue were carried out. Four weeks old C57BL/6J mice were divided in three groups: 1) mice fed a standard diet (STD) for 18 weeks; 2) mice fed an HFD for 18 weeks; 3) mice fed an HFD for 10 weeks followed by 8 weeks of HFD diet containing KlamExtra (HFD+AFA). Our results showed that the AFA diet significantly reduced body weight gain compared to the untreated obese control, whereas no change in food intake was observed. Moreover, the HFD dependent-increase of plasma triglycerides, adipocyte hypertrophy, and widespread steatosis/ballooning hepatocyte degeneration were improved by microalgae treatment. AFA administration also ameliorated HFD-induced glucose dysmetabolism, by reducing fasting glycaemia and insulinemia, and by improving glucose and insulin tolerance. Also insulin-resistance was counteracted by the AFA treatment, as indicated by the significant reduction of HOMA index in obese mice fed HFD+AFA. Moreover in liver and visceral adipose tissue of HFD+AFA mice a significant reduction in reactive oxygen species levels, TNF-α and IL-6 gene expression, NfkB and TNF-α protein levels were observed.
The results of the present study suggest that AFA supplementation is able to counteract obesity-related dysfunctions by positively modulating HFD-dependent inflammation and oxidative stress conditions.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Translational Readthrough Inducing Drugs (TRIDs): qualitative and quantitative determination of a PTC124 derivative in mice models.

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Nonsense mutations are responsible for about 10% of Cystic Fibrosis (CF) cases worldwide, introducing a premature termination codon (PTC) in the mRNA and resulting in the production of a truncated CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein[1]. The suppression therapy by Translational Readthrough Inducing Drugs (TRIDs), to restore the expression of the protein, is a promising therapeutic approach for nonsense mutations. Recently three new TRIDs (NV848, NV914, NV930) have been proposed and validated by several assays.[2,3] These molecules do not affect the Normal Termination Codon (NTC), as proved by not finding elongated proteins. Here, we report the *in vitro* preliminary study, to determine the metabolic stability of 3-acetylamino-5-methyl-1,2,4-oxadiazole, NV848, whose synthesis has been performed as reported in the literature[4], and an *in vivo* experiment to determine the biodistribution of NV848.
For the *in vitro* test: Human liver microsomes have been used for the experiment and later analysed by High Performance Liquid Chromatography (HPLC) analysis. For the *in vivo* test: Mice have been administered with NV848 and sacrificed by cervical dislocation. The organs have been collected, processed and analysed by HPLC analysis. HPLC analyses have been run on triplicates and duplicates for the calibration curve and the experiments, respectively. Moreover, a recovery test has been performed for the plasma and each organ in order to estimate the efficiency of the extraction method.

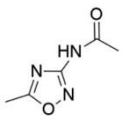

Figure 1: NV848.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Indicaxanthin and a mixture of plants sterols inhibit eryptosis induced by cigarette smoke extract

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Cell death program of red blood cells (RBCs), called eryptosis, is characterized by activation of caspases and scrambling of membrane phospholipids with externalization of phosphatidylserine (PS). Excessive eryptosis is implicated in many inflammatory pathologies and is associated to endothelial cell injury and thrombosis. It has recently been reported that cigarette smokers have high levels of circulating eryptotic erythrocytes [1] and a possible contribute of eryptosis to the vaso-occlusive complications associated to cigarette smoke has been postulated. In this study we demonstrate how the phytochemical Indicaxanthin (IND) and a mixture of plant sterols (MPS), at plasma concentrations reached after ingestion of four fruits of *Opuntia ficus-indica* (L.Mill) [2] or a drink enriched with MPS [3], inhibit eryptosis induced by whole cigarette smoke extract (CSE) or by its particulate (pCSE) or water-soluble gas (gCSE) fractions. Isolated RBCs were exposed for 4 hours to CSE, pCSE (1-2 µg nicotine/mL) or gCSE (20%). Compared to untreated RBCs, exposure to CSE or pCSE caused an increase of the levels of PS outsourcing, ceramide production, cleaved forms of caspase 8/caspase 3 and phosphorylated p38 MAPK. gCSE was unable to cause eryptosis. When RBCs were treated with CSE or pCSE in the presence of IND from 1 to 5µM or 22µM MPS, a significant dose-dependent reduction of the measured hallmarks of apoptotic death was evident. The mechanism of inhibition of the CSE-induced eryptosis by IND and MPS is currently under investigation in our laboratory. Present data indicate that particulate components are responsible of the eryptotic effect of the CSE, and prompt to formulate a new supplement containing IND and MPS to counteract possibly vaso-occlusive complications in smokers.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

The study of functional traits in populations of *Brassica rapa* L. and *Brassica oleracea* L. under drought stress conditions

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The identification of variability and adaptive traits is a valuable tool for tackling the climate changes taking place in the Mediterranean area and thus contributing to an agriculture based on the enhancement of biodiversity and of Crop Wild Relatives [1].
The BrasExplor project "Wide exploration of genetic diversity in Brassica species for sustainable crop production", funded by the PRIMA programme, aims to explore and study the genetic diversity of two species: *Brassica rapa* L. (turnips) and *Brassica oleracea* L. (cabbages), including both cultivated varieties, local populations and wild relatives [2]. A large number of populations of these species have been collected in different Countries along a wide climatic gradient in the Mediterranean area. A selection of these genotypes was cultivated in the field and in the greenhouse at the Botanical Garden of Palermo. Germination, survival, leaf and root morphometric characters and phenology up to flowering and fruiting were studied. To study the response of the genotypes in dry conditions, potted plants were subject to drought stress and recovery and a set of functional traits (leaf stomatal conductance, chlorophyll content and photosynthetic efficiency) were measured. Both *B. rapa* and *B. oleracea* genotypes showed a reduction in stomatal conductance and photosynthetic efficiency after 5-8 days of drought stress. In both species there were more sensitive genotypes and less sensitive ones. A high variability in recovery was also evident, depending on the genotype and the parameter measured. The measurement of stomatal conductance and chlorophyll fluorescence were confirmed as useful tools for evaluating the diversity of responses of different populations of *Brassica* to water stress.

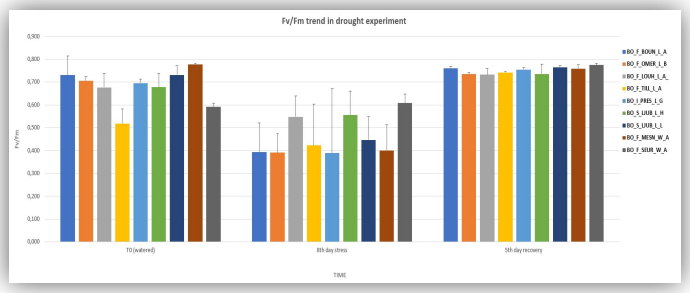

Figure. Photosynthetic efficiency assessed by chlorophyll fluorescence in populations of B. oleracea.

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RADIOTHERAPY AND BIOLOGIC EFFECTS OF RADIATIONS

[1,2] Minafra L, [1]Bravatà V, [1]Calvaruso M, [1]Pucci G, [1]Cammarata FP, [1]Stefano A, [1,2] *Forte GI and [1]Russo G.

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The Cefalù Unit research team of IBFM-CNR aims at studying the biological effects of ionizing radiations produced by conventional (*electrons, X, γ rays*) or other type of beams (*protons, laser driven or FLASH beams)*, used alone or in combination with radiosensitizers. The main goal is to develop innovative radiotherapy treatments for solid tumors, to overcome radioresistance mechanisms and going towards personalized treatments planning.
This radiobiological research is conducted with the integration of interdisciplinary skills, consisting of biologists, medical physics, computer engineers. Then, three different contributes features the radiobiological studies:

a. Cellular and molecular biology, to describe cell survival; biochemical and molecular pathways featuring the response to ionizing radiations; animal science to perform *in vivo* studies.
b. Physical-medical contribution for the dosimetric and beam simulation aspects, useful for the configuration of irradiations, as well as for the results modeling;
c. Computer engineering contribution for the imaging analysis (PET/CT), necessary for monitoring the effectiveness of treatments on small animals.In particular, in the vast field of radiobiology, the main active research topics at the Cefalù unit are:

- Radiobiological characterization studies on *in vitro* models, subjected to different types of beam (photons, protons), by the definition LQ model and α/β parameters derivation, as well as by the description of the genomic response to RI. Use of animal models for the evaluation of RBE, TCP/NTCP window of the various irradiation configurations, supported by the use molecular imaging with μPET/CT.
- Personalization of radiotherapy treatments, to define administration protocols of different total dose to the various molecular subtypes of breast cancer.
- Development of innovative combined treatments by the use of radiosensitizers, in order to enhance the radiotherapy efficacy and overcome hypoxia induced radioresistance.
- Space Radiobiology studies to characterize microgravity and radiation combined effects on tumor models for oncological therapy applications.

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

The Ri.MED Structural Biology and Biophysics Platform

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The Ri.MED Structural Biology and Biophysics group aims at providing biophysical and structural information of biological phenomena guided by the folding, aggregation and interaction of proteins, with the ultimate goal of understanding the molecular mechanisms at atomic level underlying still uncurable pathologies. The group also provides crucial support to small molecules-based drug discovery processes, development of therapeutic antibodies, and development of recombinant protein vaccines. To this aim, an interdisciplinary approach is used, which combines cutting-edge biophysical techniques (such as nuclear magnetic resonance, calorimetry, interferometry and X-ray crystallography) complemented by consolidated technical and methodological expertise in molecular biology and protein science. The integrated use of this variety of biophysical and biochemical techniques allows the characterization of the intrinsic properties of target proteins, their complexes, and the interactions in which they are involved, thus guiding in the understanding of molecular mechanisms underlying still uncurable pathologies, and in the conception of possible intervention strategies.

The group carries on several research projects in several therapeutic areas such as Neurodegenerative diseases, Cancer, Infectious diseases and Biomedical application. The diversity of all the active research projects well represents the potential of Structural Biology which can be applied to basic research as well as translational science, and can support transversally several research activities.

Nanocomposites for the development of self-cleaning and antibacterial surfaces with applications in the field of biosecurity.

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One of the ways to contract Covid-19 is through contact with contaminated surfaces. Such surfaces expose people to risks that are difficult to control and they also constitute a way not only for the COVID pandemic to spread but also for other infections, which originate from both viruses and bacteria1. One of the aims posed by researchers is to develop biosecurity materials that reduce the risk of contagion through inanimate surfaces. In this context, we focused on the development of a formulation, either as spray or paint, that would generate, upon solvent evaporation, a stable film capable of adhering to various surfaces with self-cleaning properties2. The film, a nanomaterial-based composite, would have optimized biocidal efficiency against a wide range of microorganisms. In this preliminary study, the films were fabricated with 1 and 5 % w/w of zinc oxide (ZnO) nanopowders incorporated into a polymeric matrix through a casting process. The structure and properties of the film, without ZnO nanopowders, were investigated by X-ray Photoelectron Spectroscopy (XPS) and Attenuated Total Reflection – Fourier Transform Infrared (ATR-FTIR) spectroscopies. UltraViolet-Visible (UV-Visible) and Attenuated Total Reflection – Fourier Transform Infrared (ATR-FTIR) spectroscopies, X-Ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) have been performed to characterize the obtained ZnO nanopowders. The photocatalytic activity of the films was investigated by monitoring the degradation of methylene blue in an aqueous suspension. As a control, the photodegradation activity of ZnO was also evaluated.
In future studies, we expect to improve supramolecular copolymer synthesis and explore the biocidal activity of synthesized films through in vitro tests on bacterial models such as *Staphylococcus aureus* and *Escherichia coli.*

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Young Researcher Workshop
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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Optimization of starch-based edible and biodegradable films for food preservation

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The need to extend the shelf-life of packaged food has brought research into innovative solutions. Moreover, the changing consumers' demands make "packaging" a constantly evolving field [1]. The current trend is addressing research towards the development of innovative solutions for low environmental impact packaging able to preserve and improve food features [2]. Starch is a pivotal natural polymer that can be considered a renewable resource [3]. Here, mixtures of sodium citrate or citric acid and polyethylene glycol 200 (PEG200) at various weight ratios were used to plasticize starch. Structure, interactions, and thermal properties of plasticized starches were investigated by Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) and Solid-state Nuclear Magnetic Resonance (ss-NMR) spectroscopies, alongside Thermogravimetric analysis (TGA). Compared to a single plasticizer, co-plasticizers with appropriate proportions are more effective in hindering the retrogradation of starch film. Moreover, the degree of ordered structure can affect the mechanical properties of films. Specifically, ATR-FTIR and NMR spectroscopies revealed the ability of co-plasticizers to associate with starch affecting the film's thermal stability. The technological potential of starch films was improved by enriching with the plasticizers mixture of a microemulsion containing citrus essential oil (CEO). Indeed, the latter has antimicrobial and antioxidant properties that should increase the food shelf-life. CEO is a food additive, often used as an antimicrobial ingredient, whose function is to reduce interactions with other food components, oxygen, and moisture in the environment [4]. All these factors cause the browning of fresh-cut fruits. Some films maintained the quality of fresh-cut apple slices during storage and consequently could find application in the food and packaging industry.

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COGNOME	INIZIALE NOME	CONTRIBUTO (O=ORALE, P=POSTER)
Abbate	L	O7
Abenavoli	M.R	O7
Abruscato	G	O13, P1
Acuto	S	O19
Affranchi	F	O11, P2
Albrandi	P	O7
Alcaro	S	P4,P5
Alduina	R	O1,P11,P32
Alfano	C	P49
Allegra	M	O11, O15, P46
Amata	S	P42
Amato	A	O15, P44
Andrei	J	P5
Aprile	C	P24, P40
Arcidiacono	F	P50
Arculeo	M	O3
Arizza	V	O13, P1, P3
Armetta	F	O18
Arrabito	G	P50
Attanzio	A	O11, O15, P3, P46
Auteri	M	P35
Badalamenti	R	O13, P3
Barberi	G	O16
Barra	V	O5, O17, P22, P36,
Barraja	P	P4, P5, P17, P38
Barreca	M	P4, P5, P17, P38
Belfiore	E	O6
Berger	W	P38
Bertoni	F	P17
Biscari	G	O16
Bivacqua	R	P4, P5, P17
Bivona	G	P18
Bono	A	P43
Bonsignore	R	O12
Borrelli	A	P4
Bravatà	V	P48
Bruno	M	P9
Buscaino	G	P14
Buscemi	S	P42
Buttacavoli	M	P26, P39
Caldiero	C	O7
Calvaruso	G	O11, P2

Calvaruso	M	P48
Calvi	P	O15, P44
Calvino	M.M	P51
Cammarata	F.P	P48
Campisciano	V	O10
Campora	S	O9, P18
Cancemi	P	P15, P26, P31, P39
Capri	F.C	P11, P15
Caradonna	F	P19, P25
Cardella	C	O19
Cardinale	P.S	P25
Carlisi	D	O11, P2
Carollo	P.S	P6, P12, P22
Cascione	L	P17
Catania	V	P27
Cavalieri	V	O7, O19
Cavallaro	G	P10, P20, P51
Celesia	A	P2
Chiarelli	R	P1, P7
Chiavetta	R.F	O5, O17, P36
Chillura Martino	D.F	O2, P50, P51
Ciani	R	P8
Ciaramitaro	V	P51
Cicio	A	P9
Cilla	A	P46
Cinà	G	P10
Citarrella	M.C	P27
Colige	A	P12
Conte	P	P51
Corrao	F	P6, P12, P45
Cruciata	I	P13, P25, P35
De Caro	V	O6
D'Amico	C	P15
D'Anna	F	P23, P31
D'Anneo	A	O11, P2
De Blasio	A	O11, P2
De Rosa	M	P33
De Simone	G	P33
De Vita	C	P14
Delvenne	P	P12
Di Cara	G	P31
Di Leonardo	A	O5, O17, P6, P36, P45
Di Leto	Y	P11, P32
Di Liberto	D	O11, P2

Di Liegro	C.M	P18, P34
Di Liegro	I	P34
Di Prima	G	O6
Di Stefano	V	P1
Dispenza	C	O9
Emanuele	S	O11, P2
Emili	C	P8
Faddetta	T	O7
Feo	S	P15, P26
Ferrara	V	P29
Ferraro	C	P11, P32
Fiduccia	I	P12, P45
Fiorica	C	O16
Foderà	V	P29
Forte	G.I	O19, P48
Gaglio	R	P16
Galietta	L.J.V	P4
Gallo	G	O7, P19
Gallo	A	P11, P32
Garcìa-Llatas	G	P46
Garcìa-Lopez	E.I	P41
Gargano	S	O5, P22
Gaudio	E	P17
Genco	A	P41
Genovese	M	P4
Gentile	C	P16
Geraci	A	P47
Geraci	F	P7, P39
Ghersi	G	O9, P18
Giacalone	F	O10, P24, P40
Giammona	G	O16
Giardina	I.C	P46
Giuffrida	A	P17
Giuliano	M	O11, P2
Grimbacher	B	P12
Gruttadauria	M	O10, P24, P40
Guidone	D	P4
Iemmolo	M	P18
Ilardi	V	P9
La Monica	G	P43
La Rosa	L	P16
La Scala	S	P19
Labbozzetta	M	P28
Lauria	A	P16, P43

Lauricella	M	O11, P2
Lazzara	G	P10, P20
Lazzara	V	P1
Lentini	L	P6, P12, P45
Leone	F	P20
Librici	C	P51
Licciardi	M	P29, P37
Lo Buglio	G	P18
Lo Meo	P	P51
Lo Pinto	M	O7
Luparello	C	P1, O13
Mannino	G	P16
Mannino	A.M	P21
Marcì	G	P41
Marino	S	P15
Marrone	F	O3
Martino	S	O5, P22
Martino	C	P7
Martorana	Annalisa	O16
Martorana	Annamaria	P43
Marullo	S	P23, P31
Massaro	M	O14, P8, P10, P20
Massaro	A	P46
Mauro	M	O13, P1, P3, P14
Melfi	R	P12, P36, P45
Mercati	F	O7
Migliore	M	P30, P36
Minafra	L	P48
Monaca	E	P49
Montalbano	A	P4, P5, P17, P38
Morando	M.A	P49
Morena	A	P24
Moutschen	M	P12
Mulè	F	O15, P44
Musante	I	P4
Naselli	F	P25
Nicosia	A	P18
Notarbartolo	M	P10, P20, P28
Notaro	A	O11, P2
Oddo	E	P47
Pace	A	P6, P12, P13, P35, P42, P45
Palumbo	F.S	O16
Palumbo Piccionello	A	O7, P3, P42

Pantò	F	O20
Peri	E	P26
Perricone	U	P33
Perriera	R	P6, P12, P45
Petta	E.M	P27
Piacenza	E	O2, P50, P51
Pibiri	I	P6, P12, P42, P45
Piccoli	T	P18
Pignataro	B.G	P50
Pitarresi	G	O16
Polito	G	O7
Poma	P	P28
Pratelli	G	P2
Prato	M	P10, P20
Presentato	A	O1, P11, P32
Pucci	G	O19, P48
Pucci-Minafra	I	P26
Puleo	G	P29
Punginelli	D	P1
Quatrini	P	P13, P19, P27, P35
Ragusa	M.A	P30
Raia	G	P23, P31
Raimondi	M.V	P4, P5, P17, P33, P38
Reina	C	O7
Renda	M	P4
Renzone	G	O7
Restivo	I	P3, P46
Riela	S	O14, P8, P10, P20
Rinaldi	A	P17
Rizzo	C	O4, P42
Roccheri	M.C	P7
Romeo	I	P5
Roz	E	P26
Russo	G	P48
Sabbatella	R	P49
Saladino	M.L	O18
Salemi	G	P39
Sampino	A	P11
Sampino	A.M	P32
Sancataldo	G	P29
Sànchez-Espejo	R	P8
Sardo	I	P33, P38
Scaffaro	R	P27
Scaloni	A	O7

Scancarello	A	O5, P36
Schiera	G	P18, P34
Scirè Calabrisotto	L	P13, P35
Scudieri	P	P4
Scudiero	R	P7
Serio	R	P9
Serio	G	P16
Settanni	L	P3, P16
Silvestri	A	P10, P20
Snoeck	R	P5
Sollazzo	M	P49
Spanò	V	P4, P5, P17, P38
Spinello	A	O8
Stefano	A	P48
Tarantelli	C	P17
Tarantino	G.R	P47
Terracina	F	P37
Terzo	S	O15, P44
Tesoriere	L	O11, O15, P2, P46
Titoli	S	O5, P36
Titone	G.M	P38
Tornatore	E	O1, P11, P32
Tornetta	G	P30
Tutone	M	P6, P12, P33, P45
Vaccaro	E	O7
Vaglica	F	P39
Vago	R	P8
Valentino	L	P40
Vazzana	M	O13, P1, P3, P14
Vecchioni	L	O3
Venturini	A	P4
Vetri	V	P29
Viseras-Iborra	C	P8
Vitale	F	O2
Vitale	E	P6, P12
Vizzini	A	O13, P1, P3
Volpes	S	P25
Zizzo	M.G	P6, P9, P45