3° WORKSHOP

I CHIMICI PER LE BIOTECNOLOGIE
Milano, 13-14 Febbraio 2020

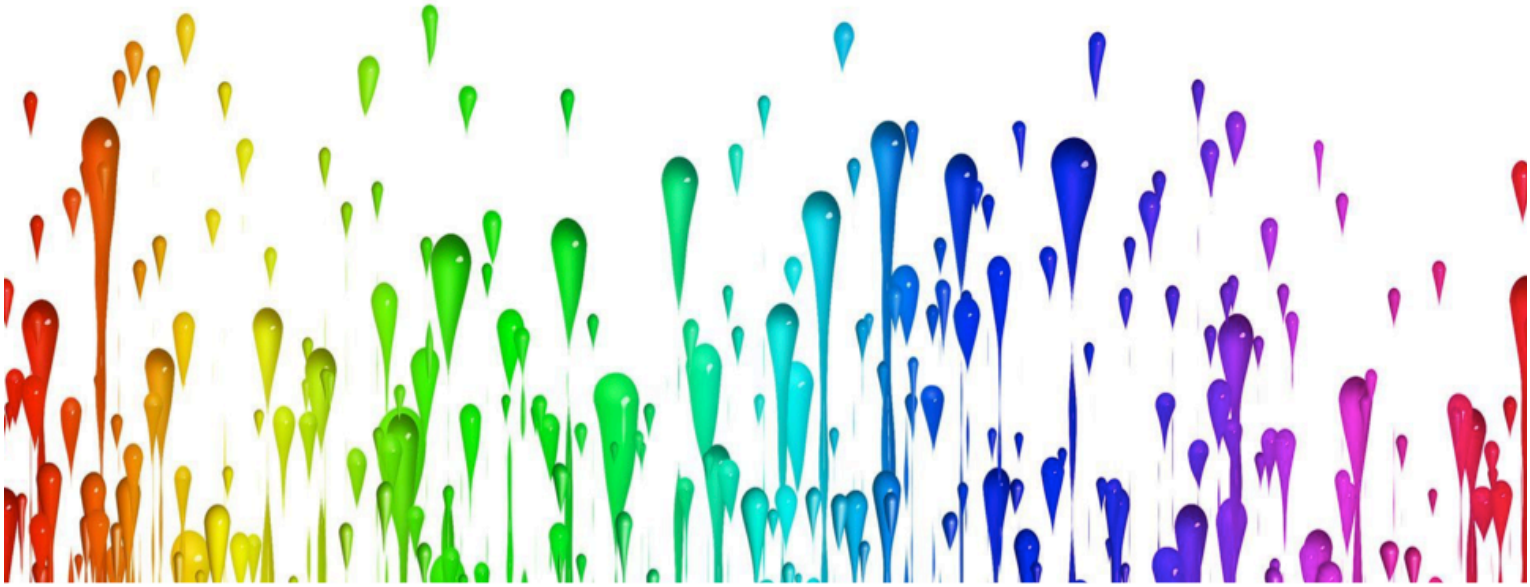
Università degli Studi di Napoli Federico II

Aula Magna–Centro CESTEV

Palazzo delle Biotecnologie

Via De Amicis 95, Napoli

ATTI DEL CONVEGNO



Società Chimica Italiana
Gruppo Interdivisionale
Biotecnologie

Gruppo Interdivisionale di Biotecnologie
Società Chimica Italiana

3° Workshop

I CHIMICI PER LE BIOTECNOLOGIE
Napoli, 13-14 Febbraio 2020

Università degli Studi di Napoli Federico II
Via Tommaso De Amicis 95, **AULA MAGNA**

PROGRAMMA

Giovedì 13 Febbraio 2020 - Apertura del convegno

12.30-13.30 Accoglienza partecipanti

13.30-13.45 Apertura Lavori: saluti di benvenuto (L. Cipolla/ Comitato Scientifico/Direttivo GIB)

Prof. M. V. D'Auria (Presidente Divisione Chimica Organica SCI), Prof. G. Piccialli (Direttore CESTEV)

13.45-16.20 Comunicazioni Orali (Chairs: Oreste Piccolo/Roberto Corradini)

13.45-14.05 ***Cosimo Risi,*** Ambasciatore "*The New Green Deal della Commissione europea"*

14.05-14.25 **Mario Malinconico**, Istituto per i Polimeri Compositi e Biopolimeri – CNR "*Poli(idrossialcanoati): da biopolimeri a bioplastiche*"

14.25-14.45 **Principia Dardano**, Materias srl, IMM-CNR "*Microneedles devices: painless theranostics tools*"

14.45-15.05 **Luca Iovine**, Centro Colture Sperimentali "*Le biotecnologie microbiche il futuro della natura*"

15.05-15.25 **Riccardo Motterle**, Head Biotechnology Development F.I.S. - Fabbrica Italiana Sintetici S.p.A. Vicenza "*FIS approach to industrial biocatalysis*"

15.25-15.45 **Silvia Scaglione**, React4Life "*Tessuti umani 3D in un sistema fluidico brevettato (MIVO) per migliorare l'affidabilità di test in vitro di prodotti farmaceutici, dermo-cosmetici e nutraceutici*"

15.45-16.20 **Flash Presentations**

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16.20-17.05 *Coffee Break & Happy Hour Poster Session*

17.05-18.25 Comunicazioni Orali (Chair: Giorgia Oliviero)

17.05-17.25 **Luisa Torsi**, Università degli Studi di Bari "*Single-molecule sensing of biomarkers*"

17.25-17.45 **Monica Terracciano**, Università degli Studi di Napoli Federico II "*Chemical strategies for the development of innovative hybrid complexes for human diseases diagnosis and treatment*"

17.45-18.05 **Pierluigi Rippa**, Università degli Studi di Napoli Federico II "*Dalla ricerca al mercato: business model design per le imprese biotech"*

18.05-18.25 **Carlo Diaferia**, Università degli Studi di Napoli Federico II "*Nanostructured active optical peptide materials***"**

18.25-19.00 Riunione dei Soci

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Venerdì 14 Febbraio 2020

8.30-11.40 Comunicazioni Orali (Chair Laura Cipolla)

8.30-8.50 **Antonio Marzocchella** Presidente della Conferenza Nazionale dei CdS In Biotecnologie, Università degli Studi di Napoli Federico II *"L'offerta formativa Biotecnologica delle Università Italiane"*

8.50-9.10 **Antonella Carillo**, Fi.Bio., ANBI *"I Biotecnologi nell'era delle biotecnologie, problematiche ed opportunità"*

9.10-9.30 **Antonella Cavazza,** Università di Parma **"***From nature to nature: valuable by-products as re-source for green and sustainable packaging*"

9.30-9.50 **Daniele Bianchi**, Eni S.p.A. Renewable Energy and Environmental R&D Center "*Microbial oils as advanced feedstock for Hydrogenated Vegetable Oil (HVO) based biorefineries*"

9.50-10.10 **Maria Maddalena Calabretta**, Università di Bologna "*Improving ATP sensing with mutated firefly luciferase and smartphone detection"*

10.10-10.30 **Caterina Carraro**, Università di Padova, "*Rediscovering classical and sustainable chemotherapeutics: how to sharpen the anticancer potential of new leads with multi-omics"*

10.30-10.50 **Massimiliano Gaeta**, Università degli Studi di Catania "*Porphyrin self-assembly in water mediated by polypeptides and calix [4]arenes*"

10.50-11.10 **Alessandra Gasparini** Università di Parma *"Processing induced lactosylation on milk whey proteins: synthesis of lactosylated epitopes for the evaluation of the effects on allergenicity and intestinal absorption*"

11.10-11.30 **Sara Battista**, Università degli Studi dell'Aquila "*Polydiacetylene liposomes containing 5-fluorouracil derivatives as sensors for the dosage of tumour biomarker enzymes"*

11.30-11.50 **Rebecca Pogni**, Università di Siena *"FISH chitinolytic biowastes FOR FISH active and sustainable packaging material:the FISH4FISH EU project"*

11.50-12.30 *Brunch*

12.30-13.30 Comunicazioni Orali (Chair: Alessandra Romanelli)

12.30-12.50 **Antonio Buonerba**, Università degli Studi di Salerno "*Photothermal therapy of cancer cells by near infrared multiphoton absorption and quenching with dansylated-glutathione coated spherical gold nanoparticles aggregates*"

12.50-13.10 **Biagio Naviglio**, Presidente dell'Ordine Regionale dei Chimici e dei Fisici della Campania "*Biotechnology for the sustainable production of the tanning process*"

13.10-13.30 **Maria Laura Alfieri** Università degli Studi di Napoli Federico II *"Gelatin-based systems for the controlled release of labile natural compounds with potent antioxidant activity"*

13.30- 14.00 Chiusura dei lavori (L. Cipolla/Direttivo G.I.B.)

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Giovedì 13 Febbraio 15.45-16.20 Flash Presentations

Concetta Avitabile, Institute of Crystallography, CNR Bari "*Self-assembly of gc PNA-diphenylalanine building blocks*"

Jessica Costa, Università di Siena "*Comparison of two different synthetic routes of magnetic Fe3O4 nanoparticles for enzymatic immobilization*"

Francesca Greco, Università degli Studi di Napoli Federico II "*Development of an innovative biosensor for diagnosing Chronic Lymphocytic leukemia: A PNA probe con detect CD5 biomarker in vitro*"

Laura Montali, Università di Bologna "*Orthogonal bio-chemiluminescent paper-based biosensor for acetylcholinesterase activity detection"*

Rosalba Moretta, IMM-CNR "*Multiparametric porous silicon optical biosensor for brugada syndrome diagnosis***"**

Vera Muccilli, Università di Catania "*Natural polyphenols and synthetic analogues as digestive enzymes inhibitors: potential antidiabetic and antiobesity agents*"

Francesco Sansone, Università di Parma "*Delivery of peptide nucleic acids using guanidiniumcalix[4]arenes as vectors*"

Alice Sosic, Università di Padova "*At the interface between Chemistry and Biology: targeting RNA with dipeptidyl-anthraquinone conjugates to develop new potential anti-HIV-1 agents*"

Indice degli abstracts *(in ordine alfabetico per primo autore)*

Gelatin-based systems for the controlled release of labile natural compounds with potent antioxidant activity

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In recent years several natural compounds have been tested for the topical treatment of skin disorders by use of a variety of transcutaneous delivery systems**.** Among hydrophilic delivery systems gelatin,[1,2] the product of collagen hydrolysis, has primarily been explored because of consolidated safe use in a wide range of medical applications, the simple fabrication methods, inherent electrostatic binding properties, and proteolytic degradability. In addition, gelatin versatility allows the design of different carrier systems, spanning from micro or nanoparticles, to fibers and hydrogels. Several reports have described the ability of gelatin hydrogels to trap bioactive molecules and/or drugs into the polymer network, thus allowing their controlled release, *e.g.* for pain treatment and wound healing and tissue engineering applications. The possibility to crosslink gelatin with synthetic and natural compounds has additionally greatly expanded the application range of this material allowing to proper tuning the mechanical properties, swelling behavior, thermal properties and other physiochemical properties.

In the present work we have investigated the ability of gelatin-based hydrogels of incorporating and releasing under controlled conditions 5,6-dihydroxyindole-2-carboxylic acid (DHICA), a key intermediate in the biosynthesis of eumelanins, the main epidermal human pigments. DHICA antioxidant profile characterized in *in vitro* assays suggested that it may act as a diffusible protective mediator under oxidative stress conditions, while studies on primary cultures of human keratinocytes disclosed its remarkable protective and differentiating effects.[3,4] In spite of these data suggesting that the indole could play an important role in preventing and treating inflammatory skin pathologies related to oxidative stress, several limitations stem from the ease of this compound to undergo oxidation with consequent loss of its properties. The methyl ester of DHICA, MeDHICA, was also tested in view of its higher stability and different solubility profile.

In addition to porcine skin type A gelatin, two modified cross-linked gelatins obtained using a bifunctional agent (*CL gelatin 1*) and chitosan (*CL gelatin 2*) were prepared that differed in the swelling behavior, showed high mechanical strength at physiological temperatures and well defined morphology. The extent of incorporation into all the gelatins tested using a 10 % indole to gelatin ratio was very satisfactory ranging from 60 to 90 % for either indoles. The kinetics of release of the indoles under physiological conditions (pH 7.4 and 5.5 at 37°C) was evaluated over 72 h by repeatedly refreshing the medium after the first hour. The highest values were obtained with *CL gelatin 1* and *2* for MeDHICA (90 % after 4 h), but a sustained release was observed for DHICA reaching 30 % at 72 h for gelatin at pH 7.4 and 40 % at pH 5.5. DHICA incorporated into *CL gelatin 1* proved fairly stable over 6 h over which time period the free indole at the same concentration was almost completely oxidized. The antioxidant power of the indole slowly released from gelatins was assayed by conventional chemical tests and even after prolonged storing in air the gelatins loaded with the indoles maintained over days the antioxidant potency, suggesting that the materials could be prepared in advance with respect to their use without alteration of their efficacy.

1. M. Foox, M. Zilberman, *Expert Opin. Drug Deliv.* **2015,** *12,* 1547-1563.
2. A. Sgambato, L. Cipolla, L. Russo *Gels*, **2016,** *2*, 28.
3.Panzella, L.; Napolitano, A.; d'Ischia, M. *Pigment Cell Melanoma Res.*, **2010**, *24*, 248-249.
4. Kovacs, D.; Flori, E.; Maresca, V.; Ottaviani, M.; Aspite, N.; Dell'Anna, M.L.; Panzella, L.; Napolitano, A.; Picardo, M.; d'Ischia, M. *J. Invest. Dermatol.*, **2012**, *132*, 1196-1205.

Polydiacetylene liposomes containing 5-fluorouracil derivatives as sensors for the dosage of tumour biomarker enzymes.

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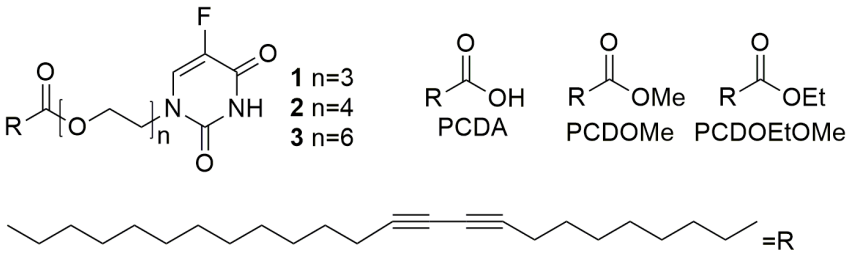
5-Fluorouracil (5-FU) is a strong chemotherapeutic agent largely used in the treatment of solid tumors (such as breast, colon, and skin cancer).[1] Among its target enzymes there are thymidylate synthase, thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase. 5-FU has a very narrow therapeutic window and about 75% patients are not treated with the correct dosage of the drug. This aspect causes severe toxic effects or reduced therapeutic efficacy: [2] about 15% of patients are overdosed whereas about 60% of patients are underdosed. Currently it is not possible to detect and measure these biomarker enzymes exploiting easy, fast and cheap methods at the aim to arrive to a personalized dosing. Based on these premises, polydiacetylene (PDA) surfactants functionalized with 5-FU on the surface were prepared to be used as components of liposomes-based sensors for TP enzyme, one of the of 5-FU targets commercially available. These surfactants bear an *ene-yne* moiety in the tail that allows them to polymerize if irradiated with UV light at 254 nm for few minutes. The obtained system shows a high conjugation extent that confers a color to the solution (normally blue). This structure makes the aggregates very sensitive to external stimulations; if for example a target molecule can interact with a moiety on the vesicles surface or if happens a variation of pH or temperature, the conjugation can become less extended or can be interrupted causing a colorimetric variation of the solution investigated.[3] For these reasons, liposomes containing 10,12-pentacosadiynoic acid (PCDA) and a 5-FU derivative with or without natural phospholipids (1,2-dioleoyl-*sn*-glycero-3-phosphocholine or 1,2-dimirystoyl-*sn*-glycero-3-phosphocholine) were prepared to evaluate the possible colorimetric variation upon the addition of TP enzyme. The three 5-FU derivatives, are no ionic diacetylene surfactants (**1**,**2**,**3**) differing for the polar spacer length (3, 4 or 6 oxyethylene units, respectively, Scheme 1) and were used to confer specificity to the aggregates. Also the behavior of same formulations adding bovine serum albumin (BSA) enzyme was investigated at the aim to evaluate its possible nonspecific electrostatic interaction with our system. At least we studied other factors that can influence the specificity and the sensitivity of the colorimetric variations and the binding efficacy between 5-FU and TP enzyme such as the exposition of 5-FU to the bulk solution, the presence of a component that isn't included in the polymeric bilayer and the liposome surface charge. For this last reason we prepared analogue formulations in which a part of PCDA was replaced by a synthetic PCDA ester derivative (PCDOMe or PCDOEtOMe, Scheme 1).



Scheme 1

1. Arias, J. L. *Molecules*, **2008**, *13*, 2340-2369.
2. Saif, W. M.; Choma, A.; Salamone, S. J.; Chu, E. *J. Natl. Cancer Inst*. **2009**, *101*, 1543-1552.
3. Dong, J. A.; Kim, J. M. *J. Acc. Chem. Res.*, **2008**, *41*, 805-816.

Microbial oils as advanced feedstock for Hydrogenated Vegetable Oil (HVO) based biorefineries

Daniele Bianchi

Eni S.p.A. Renewable Energy and Environmental R&D Center - Novara, Italy

La produzione di biocarburanti dovrà nei prossimi anni raggiungere obiettivi sempre più ambiziosi sia dal punto di vista quantitativo che qualitativo. La Direttiva Europea RED II ha infatti fissato un target del 14% di energia rinnovabile da introdurre nel settore dei trasporti entro in 2030, con una quota pari al 3,5% di biocarburanti avanzati, cioè prodotti da biomasse non in competizione con il settore alimentare.

Per fronteggiare questa sfida Eni ha riconvertito due raffinerie tradizionali (Venezia e Gela) in bioraffinerie, basate sul processo Ecofining per la produzione di greendiesel via idrogenazione di oli vegetali. Tali raffinerie richiederanno a regime un'alimentazione pari a 1 milione di tonnellate/anno di oli vegetali, di scarto o prodotti da fonti alternative.

Fra queste ultime, un'opzione è la produzione di oli microbici a partire da biomasse ligninocellulosiche di scarto (es. paglia di grano).

Il primo stadio del processo prevede la saccarificazione delle biomasse per produrre zuccheri cellulosici via pretrattamento fisico (steam explosion) e successiva idrolisi enzimatica.

Nel secondo stadio gli zuccheri vengono fermentati con lieviti oleaginosi in grado di accumulare all'interno della cellula fino al 70% di oli microbici, con composizione analoga a quella degli oli vegetali, e specifiche adatte per l'alimentazione nelle bioraffinerie.

Questa presentazione descrive le fasi del processo, la procedura di recovery e la caratterizzazione degli oli microbici fino all'utilizzo finale nel processo di hydrotreating.

Photothermal Therapy of Cancer Cells by Near Infrared Multiphoton Absorption and Quenching with Dansylated-Glutathione Coated Spherical Gold Nanoparticles Aggregates

Antonio Buonerba,[1]* Rosita Lapenta,[1] Magda Licasale,[1] Anna Donniacuo,[1] Elena Vezzoli,[2] Andrea Falqui,[2] Roberto Piacentini,[3] Claudio Grassi[3] and Alfonso Grassi[1]

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The development of novel drugs and materials effective in the care of cancer is enormously focusing interest of the scientific community. The photoablation of cancer cells and tissues, induced by exposure to near infrared (NIR), results particularly attractive because this radiation offers deep penetration and low level of damaging of healthy biological tissue.[1] With respect to continuous wave radiation, pulsed NIR results even preferable because it is able to selectively trigger the response of suitable multiphoton absorbers, leaving unaffected normal tissues. In this contest, engineered gold nanoparticles (AuNPs) are particularly attractive due to their chemical stability, biological compatibility and the response to NIR in the form of intense emission of light and/or heat.

In this contribution, we report on the synthesis, characterization and photochemical properties of spherical AuNPs of 2 nm protected with glutathione (AuNP-G) or glutathione functionalized with dansyl fluorophore (AuNP-DG). The simple synthetic procedure allows the recovery of nanoparticle aggregates of few hundred nanometers, which are readily internalized in 1h into neuronal cells or hepatocyte tumor cells HepG2 (Fig. 1) without affecting their viability. When irradiated with pulsed NIR at 740 nm, the cells treated with aqueous solutions of AuNP-G and AuNP-DG (7-700 μg/mL) showed an intense increase of temperature determining efficient cell death in few seconds. The photothermal effect was attributed to the fast quenching of luminescence under both UV-Vis and multiphoton NIR excitation. These results are unprecedented when compared to the behaviour of cells internalized with single spherical AuNPs-G under the same conditions.[2]

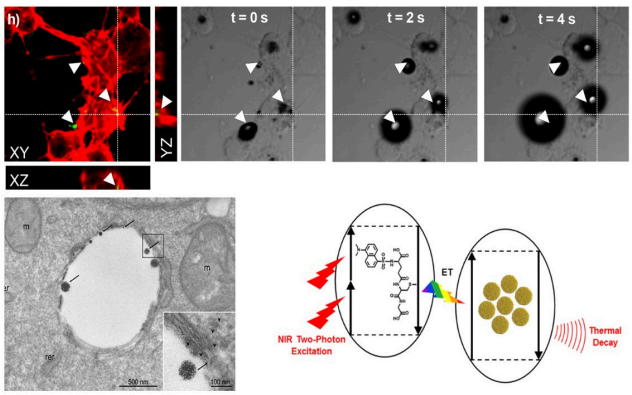

Figure 1.

1. Riley, R. S.; Day, E. S., *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2017**, 9, e1449.
2. Yu, M.; Zhou, C.; Liu, J.; Hankins, J. D.; Zheng, J. *J. Am. Chem. Soc.* **2011**, *133*, 11014.

Improving ATP sensing with mutated firefly luciferase and smartphone detection

Maria Maddalena Calabretta[1], Ruslan Álvarez-Diduk[2], Laura Montali[1], Antonia Lopreside[1], Elisa Michelini[1,3], Aldo Roda [1,3], Arben Merkoçi[2,4]

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[2]*ICN2 - Nanobioelectronics & Biosensors Group, Institut Catala de Nanociencia i Nanotecnologia, Campus UAB, Bellaterra, Barcelona (Spain).*
[3]*INBB, Istituto Nazionale di Biostrutture e Biosistemi, Roma, (Italy).*
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ATP-driven bioluminescence relying on the D-luciferin-luciferase reaction is widely employed for several biosensing applications where bacterial ATP detection allows to verify microbial contamination for hygiene monitoring in hospitals, food processing and in general for cell viability studies. Rapid ATP kit assays are commercially available, but the development of an ATP biosensor characterized by low-cost, portability, and adequate sensitivity where the reagents are immobilized is highly valuable to facilitate the early detection and rapid screening. Several attempts are aimed at improving the analytical performance of the assay by increasing luciferase stability, by improving the quantum yield of the light producing reaction by mutating the sequence encoding for the luciferase to develop newly chimeric luciferase with lower Km value for ATP and high specific activity. We used a novel luciferase called PLG2 that has enhanced specific activity, and thermal and pH stability compared to the commonly used *Photinus pyralis* luciferase. Thanks to low-cost wax printing technology and an innovative freeze-drying procedure, we developed a user-friendly, ready-to-use and stable ATP sensing paper biosensor that can be integrated in a portable light detector, including smartphone-integrated photocamera. The developed ATP sensing paper is able to quantify in 10 minutes as low as 10^{-14} moles of ATP in liquid samples. As proof-of concept, we analysed urinary microbial ATP as a biomarker of Urinary Tract Infection (UTI), confirming the capability of the ATP sensing paper to detect the threshold for positivity corresponding to 10^5 colony-forming units (CFU) of bacteria per mL of urine.[1]

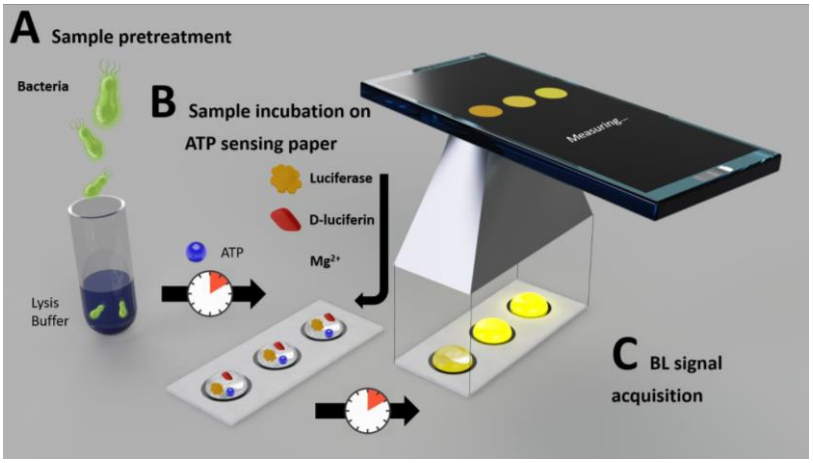

Figure 1: Schematic representation of the optimized ATP sensing paper assay.

1. Calabretta, M.M.; Álvarez-Diduk, R.; Michelini, E.; Roda, A.; Merkoçi, A. *Bions. and Bioelectr.* **2020**, *150,* 111902.

Rediscovering classical and sustainable chemotherapeutics: how to sharpen the anticancer potential of new leads with multi-omics

Caterina Carraro[a], Tim Helbing[b], Lorenzo Bonaguro[c], Alice Sosic[a], Anna Aschenbrenner[c,d], Joachim L. Schultze[c,e], Valentina Gandin[a], Richard Gottlich[b] and Barbara Gatto[a]

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[b] *Institute of Organic Chemistry, Justus Liebig University Giessen, Heinrich-Buff-Ring 17, 35392 Giessen (Germany)*

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Moving toward the era of stratified medicine, the search for novel anticancer drugs needs to crosstalk with omic sciences for the design of multi-approach personalized therapies.[1]
Recent studies on old but clinically active chemotherapeutics unearthed corollary or totally revisited mechanisms of action for these drugs. Taken for granted for too long, classical antitumor agents are going across an intense process of re-estimation of their unforeseen specificity for cellular targets and gene expression signatures, with impacting consequences on the associated toxico-pharmacologic profiles.[2] Pseudotargeting of specific cancer genotypes by nitrogen mustards has recently been reported for chlorambucil against BRCA 1/2-deficient tumors, thus demonstrating that even agents commonly known as *aspecific* could exhibit preferential cancer type toxicities.[3]
Searching for novel effective anticancer leads, we have recently identified bis-3-chloropiperidines (B-CePs) as an attractive class of sustainable mustard-based agents.[4] Surprisingly, aromatic linkers connecting the two reactive centers of B-CePs were demonstrated to unleash the activity of compounds especially against chlorambucil-resistant pancreatic cancer 2D and 3D cell cultures. As a proof-of principle of their intended mechanism of action, aromatic derivatives demonstrated to efficiently fragment isolated and cellular DNA. Nevertheless, studies are ongoing to unveil the distinctive mechanisms beyond DNA damage accounting for the unexpected tropism against the pancreatic cell line.
In this sense, we employed a multi-omic approach to elucidate cellular determinants of sensitivity for the analyzed class of compounds. RNA-seq and ATAC-seq allowed to portrait gene expression and epigenetic profiles upon stimulation with selected derivatives, in terms of differential transcriptional and chromatin states. This signature-driven approach may open new perspectives in the refinement and exploration of the mechanism of action of both classical chemotherapeutics and new chemical entities, facilitating the mindful repositioning of current and future therapeutics.

1. Matthews, H.; Hanison, J.; Nirmalan, N., *Proteomes* **2016,** *4* (3).
2. Hato, S. V.; Khong, A.; de Vries, I. J.; Lesterhuis, W. J., *Clin Cancer Res* **2014,** *20* (11), 2831-7.
3. Tacconi, E. M.; Badie, S.; De Gregoriis, G.; Reislander, T.; Lai, X.; Porru, M.; Folio, C.; Moore, J.; Kopp, A.; Baguna Torres, J.; Sneddon, D.; Green, M.; Dedic, S.; Lee, J. W.; Batra, A. S.; Rueda, O. M.; Bruna, A.; Leonetti, C.; Caldas, C.; Cornelissen, B.; Brino, L.; Ryan, A.; Biroccio, A.; Tarsounas, M., *EMBO Mol Med* **2019,** *11* (7), e9982.
4. Zuravka, I.; Roesmann, R.; Sosic, A.; Wende, W.; Pingoud, A.; Gatto, B.; Gottlich, R., *ChemMedChem* **2014,** *9* (9), 2178-85.

From Nature to Nature: Valuable By-products as *Re-source* for Green and Sustainable Packaging

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Packaging has the essential role of preserving products, preventing food deterioration, enhancing shelf-life, and allowing for food storage and distribution to different areas. In addition, packaging design conveys useful information to consumers. This critical marketing instrument plays a powerful role in promoting values and expressing the culture of a time period. Nevertheless, packaging is considered a dangerous agent capable of wreaking havoc on the environment. In particular, the diffusion of high amounts of plastic is a matter of much concern these days; and, according to recent legislation[1], there is a great need for alternative materials that will not inflict detrimental effects on the ecosystem. To this end, scientific research is looking for new packaging solutions that can prevent food spoilage and waste but possibly have a lesser impact on our ecosystem.

The best source of green and sustainable material is obviously Nature, which offers many examples of biodegradable "packaging" containing bioactive compounds able to protect food. In the agro-industrial field, enormous amounts of by-products unsuitable for commercial use due to their dimension, shape, or ripeness degree are produced. Moreover, large portions of vegetables are discarded during industrial processing, generating significant disposal costs and significant negative environmental impact. These materials are actually a useful source of valuable compounds that can be reinserted in the productive cycle, according to the Circular Economy[2] plan promoted by world politic.

As a result, innovative "active" packaging in the form of a film or spray solution has been developed through the use of natural compounds extracted from artichoke, asparagus and onion by-products. Extraction procedures have been set up, and active molecules, such as antioxidant polyphenolic compounds[3] and fructooligosaccharides with prebiotic properties,[4] have been characterised by chromatographic methods.

This "active" packaging is safe, sustainable, compostable, and even edible. Film properties such as thickness, transparency, flexibility, and flavour can be customised according to their application on different types of products. Studies on the shelf-life of meat and vegetables have showed positive effects on colour and odour stabilisation, flavour and ripening control, increment of oxidative stability, and the delay in mould development.

Indeed, extracts could have many interesting potential applications for numerous other fields, such as food technology, cosmetics, and nutrition/functional foods.

Exhausted waste after extraction, mainly constituted by cellulose and lignine, can still be used for the production of bulk materials for secondary packaging and nanocellulose, thus "closing the loop" and reaching the goal of zero-waste promoted by European Community..

1. European Parliament Commission **2018**: Single-use plastics: New EU rules to reduce marine litter.
2. European Parliament Commission **2015**: Closing the loop, an EU action plan for the Circular Economy.
3. Llorach, R.; Espin, J.C.; Tomas-Barberan, F.A.; Ferreres, F. *J. Agric. Food Chem.* **2002**, *50*: 3458-3464.
4. Corradini, C.; Lantano, C.; Cavazza, A; *Anal. Bioanal. Chem.* **2013**, *405*: 4591-4605.

Microneedles devices: painless theranostics tools

P. Dardano[1], M. Battisti[2], S. De Martino[2], B. Miranda[1,3], I. Rea[1], L. De Stefano[1]

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Modern approach to healthcare is largely mediated by economic factors since public health budget is in constant growth[1]. Many possible solutions have been proposed to face this big problem from every possible side: for example, food awareness campaigns, mass vaccinations, diagnostic screening by age group. Every large scale initiative sees its effectiveness greatly reduced by the intrinsic limits of traditional medicine, based almost exclusively on clinical practice supported by statistical studies and on pharmacological chemistry[2]. The next revolution in medicine, and more generally speaking in healthcare, will come from innovative technologies acquired from very different field of applications, such as large electronic consumer and telecommunication, and adapted to specific problems[3]. In particular, micro- and nanotechnologies, i.e. systems and devices having dimensions under 10^{-6} m, are driving the development of new products more performing than the existing ones. Scaling down the size of common materials, both the organics and the inorganics, it is not only matter of fabrication ability, but it opens the way to a completely diverse world where things behave different[4]. In some cases, the matter properties drastically change on the nanoscale. Crystalline silicon, the basic material in electronic, is not light emitting and can cause silicosis, a pulmonary disease, whereas its nanostructured counterpart, the porous silicon, is photoluminescent and completely biodegradable; carbon is highly insulating, since it has a very large bandgap, while carbon nanotubes and graphene are highly conductive; and the list of examples could continue for a long. In other cases, such as the one of popular microfluidics, geometrical features matter more than bulk properties. As a matter of fact, when linear dimension (L) decreases, surfaces are more important than volume. Moreover, due to the intrinsic difficulty of translating laboratory science into industrial production, most of the scientific discoveries found in the academic habit is lost in a "Death Valley" before industry could get advantages from them. In this view, technology transfer companies play a strategic role in overcoming the gap between scientific research and industrial development. In South Italy, Materia's S.r.l. is one of the most active start-up company highly specialized in technology transfer In this work, we will focus on a specific system, developed in collaboration with academic institutions, that is explicative of brand new technologies applied to human healthcare: the polymeric microneedles. Materia's Srl has patented two technologies for fabricating MN by using a photolithographic approach. These devices can be added with a porous silicon free-standing membrane, using, for example, polyethylene glycol (PEG) or other biodegradable polymers and a commercial photo-catalyzer[5,6,7].

1. Henderson S, et al. Health & social care in the community **2018**, 26(4): 590-603.
2. Rich CR, et al. In: Rich CR, Singleton JK, Wadhwa SS (eds.) (2nd Edn) **2018,** Routledge ed.
3. Rea I, et al. Adv. Healthcare Mater **2017,** 6(3): 1601125.
4. R Perumal, et al. Frontiers in Laboratory Medicine **2017,** 1(2): 49-54.
5. A Caliò, et al. Sensors and Actuators B: Chemical **2016,** 236: 343-349.
6. P Dardano, et al. Materials **2015,** 8(12): 8661-8673.
7. P Dardano, et al. Biomed Opt Express **2016,** 7(5): 1645-1655.

Nanostructured active optical peptide materials

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Biological architectures generated *via* multiscale self-organization of natural building blocks are inspiring tools for the development of novel synthetic nanomaterials. Combining the intrinsic biocompatibility with remarkable tenable multifunctional properties, peptides in particular are opening promising avenues for their applications in few directions.[1] For example, the field of nano-photonics has been taken advantages from a novel paradigm of peptide/protein optical photoluminescence (PL). Chemically and rationally synthesized peptides, arranged in β-sheet rich nanostructures, exhibit pronounced visible blue/green PL, which is considered as an optical signature of their common organization.[2] In line with this evidence, we report about PL phenomena detectable into a series of supramolecular nanostructures generated by aromatic poly-peptides.[3,4] The possibility to engineering the building blocks, the optimization of state of the material and the physical treatment of the self-assembled system allowed us to obtain nanofiber, filled aggregates, biodots and films showing active optical properties. All these materials are highly promising for the development of innovative tools in bioimaging, local diagnosis, biomedical light therapy and implantable optical biochips.

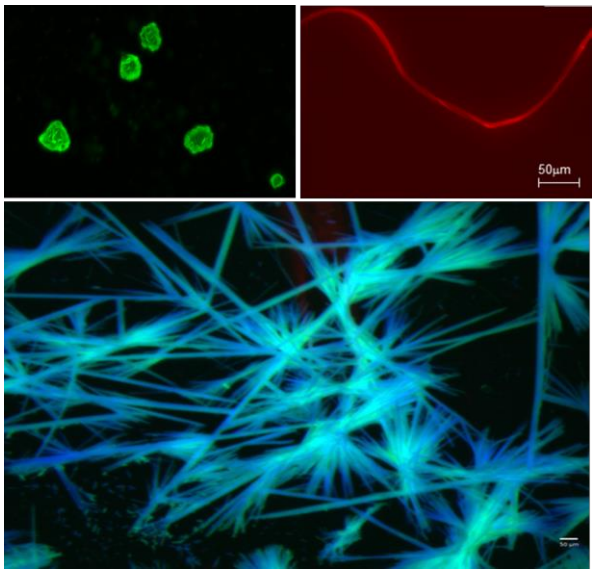

Figure 1. Fluorescence microphotos of different nanostructurated active optical peptide materials: green biodots, red fibers and blue-green crystals.

1. Tesauro, D; Accardo, A; Diaferia, C; Milano, V; Guillon, J; Ronga, L; Rossi, F. *Molecules* **2019**, *24(2),* 351-378.
2. Apter, B; Lapshina, N; Handelman, H; Faindberg, B.D; Rosenman, G. *Small* **2018**, *14(34)*, 1801147.
3. Diaferia, C; Sibillano, T; Altamura, D; Roviello, V; Vitagliano, L; Gianni, C; Morelli, G; Accardo, A *Chem- Eur. J.* **2017**, *23(56)*, 14039-14048.
4. Avitabile, C; Diaferia, C; Roviello, V; Altamura, D; Giannini, C; Vitagliano, L; Accardo, A; Romanelli, A. *Chem- Eur. J.* **2017**, *26(65)*, 14850-14857.

Porphyrin self-assembly in water mediated by polypeptides and calix[4]arenes

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The possibility to manipulate matter at the nanometric scale has been shaping our life thanks to increasing level of bio- and nano-technological devices. In perspective, self-assembly appears as powerful and attractive strategy for constructing complex nanostructures by spontaneous organization of appropriate building blocks.[1] To self-organize molecular building blocks into well-organized structures is strictly needed to control their size, dimensionality and properties. In fact, non-covalent porphyrin self-assembly permits to obtain large aggregates in aqueous media. However, the phenomenon is often ungovernable for practical applications. Several strategies have been implemented to this aim, among them, the template-assisted self-assembly represent a promising strategy.[2]

Herein, we demonstrate the possibility of controlling in water the H_2TPPS4 J-aggregation, after demetallation/protonation of its zinc(II) derivative, ZnTPPS4, at acidic conditions and in presence of poly-L-lysine, PLL, as template. By varying the polylysine degree of polymerization, we can modulate the final aggregation. Complete spectroscopic studies confirm as follows: i) if short PLLs are used, the fast demetallation/protonation of ZnTPPS4 drives the rapid formation of several families of less organized J-agg, made up of few monomers; ii) longer PLLs promote structural reorganizations of the protonated porphyrins leading to the formation of long ordered J-agg, resulting as well, in a chirality enhancement.[3]

Furthermore, a template strategy based on host-guest chemistry is also reported. In particular, calixarenes have been successfully employed in interactions with porphyrin to achieve supramolecular complexes.[4] Full spectroscopic measurements prove that calixarenes are able to control the stoichiometry, sequence, dimensionality and chirality of porphyrin self-assembly process.[5]

In this contribute we report how the dimensionality (1D, 2D or 3D) of the supramolecular porphyrin-calixarene complexes strictly depends on geometric parameters. For instance, di-topic biscalix[4]arenes drive towards the building of 1D and 2D structures, also conveying chirality if an asymmetric calixarene is used. On the contrary, we also show how tri-topic triscalix[4]arenes lead to 3D supramolecular complexes.

1. Zhang, S., *Nature Biotechnology* **2003**, *21*, 1171-1178; Whitesides, G.M.; Mathias, J.P.; Seto, C.T.; *Science*, **1991**, *24*, 1312-1319.

2. Koti, A.S.R.; Periasamy, N., *Chem. Mat.* **2003**, *15*, 369-371; Gaeta, M; Oliveri, I.P.; Fragalà, M.E.; Failla, S.; D'Urso, A.; Di Bella, S.; Purrello, R., *Chem. Commun.* **2016**, *52*, 8518-8521; Occhiuto, I.G.; Samperi, M.; Trapani, M.; De Luca, G.; Romeo, A.; Pasternack, R.F.; Monsù Scolaro, L., *Journal of Inorganic Biochemistry* **2015**, 153, 361-366.

3. Gaeta, M.; Randazzo, R.; Cristaldi, D.A.; D'Urso A.; Purello, R.; Fragalà, M.E., *Journal of Porphyrins and Phthalocyanines*,**2017**, *21*, 436-430; Gaeta, M.; Raciti, D.; Randazzo, R.; Gangemi, C.M.A.; Raudino, A.; D'Urso, A.; Fragalà. M.E.; Purrello, R., *Angew. Chem. Int. Ed.* **2018**, *57*, 10656-10660.

4. Guo, D.-S.; Chen, K.; Zhang, H.-Q.; Liu, Y., *Chemistry-An Asian Journal* **2009**, *4*, 436-445; Guo, D.-S.; Liu, Y., *Acc. Chem. Res.* **2014**, *47*, 1925-1934.

5. D'Urso, A.; Fragalà, M. E.; Purrello, R., *Chem. Commun*., **2012**, *48*, 8165–8176; D'Urso, A.; Marino, N.; Gaeta, M.; Rizzo, M.S.; Cristaldi, D.A.; Fragalà, M.E.; Pappalardo, S.; Gattuso, G.; Notti, A.; Parisi, M.F.; Pisagatti, I.; Purrello, R., *New J. Chem.* **2017**, *41*, 8078–8083.

Processing induced lactosylation on milk whey proteins: synthesis of lactosylated epitopes for the evaluation of the effects on allergenicity and intestinal absorption

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Cow's milk is listed among the big 14 food allergens. It is the most common food allergy for young children with an incidence of 2-7.5% of the population[1]. Main milk allergens are caseins (80% of milk protein content[2]) and whey proteins (16% of milk protein content[2]), in particular the most abundant β-lactoglobulin and α-lactalbumin. In order to guarantee a safety and easy of use for the consumers, milk and its derivatives undergo several technological treatments that involve the use of high temperatures and might affect milk proteins inducing structural and chemical modifications[3]. The most common is the Maillard reaction, a natural reaction that occurs in food between protein and sugar (lactose for milk) and it is catalysed by the high temperatures. It starts with the condensation of lactose with lysine residues, forming a first reaction intermediate called Amadori compound. This modification may alter properties such as protein digestibility and allergenicity, affecting the binding with the human IgE. Thus, this study aimed at studying how the lactosylation affects whey proteins and in particular the effect on the IgE binding. A screening on different UHT and pasteurized milk samples was performed. Whey proteins were extracted after casein precipitation at pH 4.6 and analysed with UPLC-MS for protein quantification. It was confirmed the increase of the lactosylation degree with the harshness of the treatment. Then, *in solution* tryptic and chymotryptic digestion was performed followed by UPLC-MS and LTQ-Orbitrap analysis for the identification of the lactosylation sites. From the comparison with the literature, it was found that some lysine residues that could be lactosylated during the thermal treatment are present in β-lactoglobulin and α-lactalbumin epitopes. Thus, four β-lactoglobulin epitopes where then selected and synthesized with the Fmoc protocol for the solid phase peptide synthesis. A procedure for the lactosylation of these epitopes was then developed. Peptides in the lactosylated and unmodified form will be used for ELISA tests to verify the effect of the presence of a sugar on the binding with the IgE. An important step to be considered for the evaluation of the allergenicity is the human gastrointestinal digestion. From *in vitro* semi-dynamic gastrointestinal digestion studies performed on whey protein concentrates following the INFOGEST standard protocol[4], peptides containing lysine residues that were found lactosylated in the former screening were identified after the gastric digestion. Peptide KIDALNENKVLVL was selected and lactosylated for transepithelial transport studies on Caco-2 cells to evaluate the possible intestinal absorption of the peptide in both the forms. It was found that the presence of lactose has a little effect on peptide absorption and peptide in the modified form is able to be absorbed.

1. Bu, G.; Luo, Y.; Chen, F.; Liu, K.; Zhu, T. *Dairy Sci. Technol.* **2013**, *93*, 211-223.
2. D'Alessandro A.; Zolla L.; Scaloni, A. *Mol. BioSyst.* **2011**, *7*, 579-597.
3. Meltretter, J.; Wüst, J.; Pischetsrieder, M. *J. Agr. Food Chem.* **2014**, *62*, 10903-10915.
4. Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carrière, F.; Boutrou, R.; Corredig, M.; Dupont, D.; Dufour, C.; Egger, L.; Golding, M.; Karakaya, S.; Kirkhus, B.; Le Feunteun, S.; Lesmes, U.; Macierzanka, A.; Mackie, A.; Marze, S.; McClemens, D.J.; Ménard, O.; Recio, I.; Santos, C.N.; Singh, R.P.; Vegarud, G.E.; Wickham, M.S.J.; Weitschies, W.; Brodkorb, A. *Food Funct.* **2014**, *5*, 1113-1124.

Le BIOTECNOLOGIE MICROBICHE il futuro della NATURA

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Abstract

I microorganismi sono stati spesso sottovalutati o addirittura visti solamente come patogeni, in realtà questa incredibile moltitudine ci aiuta a sopravvivere e collabora con noi per il mantenimento del nostro ecosistema. La C.C.S. è da anni leader nella ricerca, selezione e commercializzazione di biotecnologie microbiche utili in agricoltura, nell'ambito della BIOREMEDIATION e in un prossimo futuro anche in quello della salute umana. Nei suoi formulati ci sono efficienti microrganismi che favoriscono la nutrizione delle piante e la prevenzione ai numerosi stress presenti nei suoli agricoli, migliorando complessivamente le performance qualitative (NUTRACEUTICHE) e quantitative delle piante. O anche microorganismi che scompongono le molecole dei residui tossici presenti nei suoli o nelle acque. Inoltre si sperimentano i cosiddetti FARMACI "VIVENTI" quali soluzioni in ambito umano. I Funghi micorrizici, Actinomiceti, Funghi saprofiti, Batteri e Lieviti sono i protagonisti di questo passato divenuto presente.

MICROBIAL BIOTECHNOLOGIES the future of NATURE

Abstract

Microorganisms have often been underestimated or even seen only as pathogens, in reality this incredible multitude helps us survive and collaborates with us for the maintenance of our ecosystem. The C.C.S. For years, it has been a leader in the research, selection and marketing of microbial biotechnologies useful in agriculture, in the context of BIOREMEDIATION and in the near future also in that of human health. In its formulations there are efficient microorganisms that favor the nutrition of plants and the prevention of the numerous stresses present in agricultural soils, improving overall the qualitative (NUTRACEUTICAL) and quantitative performances of the plants. Or even microorganisms that break down the molecules of toxic residues present in soils or waters. In addition, the so-called "LIVING" DRUGS are tested as solutions in the human field. Mycorrhizal mushrooms, Actinomycetes, Saprophytic mushrooms, Bacteria and Yeasts are the protagonists of this past that has become present.

From Microbial Biopolymers to Bioplastics

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The term biopolymers refers to a broad class of materials that derive from naturally occurring resources. Biopolymers can be obtained through extraction from biomasses, but also through chemical or biotechnological methods from raw natural substrates. They are used to produce bioplastics, which could substitute fossil fuel-derived commodities. Among them, polyhydroxyalkanoates (PHAs) are polyesters synthesized by microorganisms as energy reserve. The most important member of PHA family is poly(3-hydroxybutyrate) (PHB). PHB is mechanically similar to polypropylene, even though its thermal instability, brittleness, and stiffness limit its applicability. Improving PHB physical properties can be achieved by blending it with natural additives or by-products of industrial processes. The lecture takes the form of a case study about the effects of three natural, phenol-based, and polysaccharidic compounds on PHB properties. An overall improvement of polymer processability and photostability, along with changes in crystallization rates, was observed. The study provides evidence that natural additives have the potential for promoting the transition from biopolymers to bioplastics in a sustainable way, both from an environmental and economical point of view.

FIS approach to industrial biocatalysis

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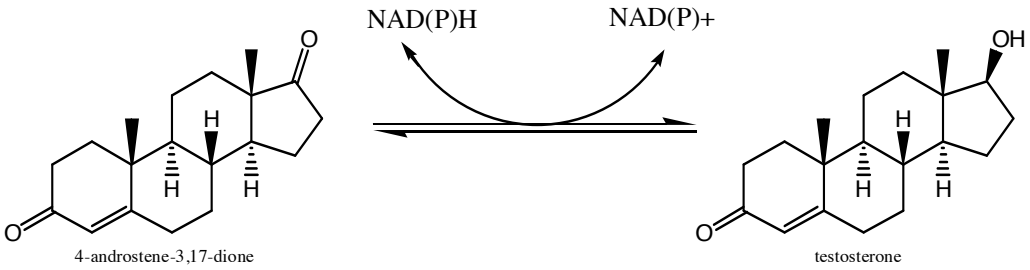
F.I.S. S.p.A. belongs to Holding F.I.S., an international and independent group of companies.

FIS is a leading pharmaceutical chemicals manufacturer offering an integrated service, from R&D to full scale production, to its Customers. FIS core business is the production of Active Ingredients, Key Intermediates and Building Blocks.

In this presentation FIS approach to Industrial Biocatalysis is presented with some examples.

The synthesis of Testosterone from Androstendione (see Scheme 1) is reported as case history [1,2]



Scheme 1

The transformation is performed using a ketoreductase developed by FIS. All the project steps:

- enzyme identification and production
- fermentation optimization and scale
- biocatalytic process setup and optimization
- CCP identification by DoE approach
- Plant scale-up

are presented.

1. Motterle, R.; Fogal, F. et al. WO2011000693

2. Motterle, R.; Fogal, F. et al. EP2449128

Biotechnology for the sustainable production of the tanning process

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The Italian tanning industry is traditionally organized in industrial districts, each of them with its own production specialization: Arzignano, Zermeghedo and Montebello in Veneto; S. Croce sull'Arno and Ponte a Egola in Tuscany, Solofra in Campania and Turbigo and Castano Primo in Lombardy. The tanning industry is essentially concerned with the transformation of a waste from the meat and / or milk industry into an industrial product suitable for use in the production of leather and / or leather articles. Therefore, leather can be considered as an environmentally sustainable solution to a real problem of disposal of large quantities of animal remains that originate from the meat industry. In fact, the availability of raw hides on the market depends on the amount of slaughtering for food purposes and is in no way influenced by the needs of the leather manufacturing industry; in essence, the number of animals reared and slaughtered at the end of their life is functional to the needs of other industries (meat, milk, wool, etc.).

The major environmental problems of the tanning industry derive from the fact that in the production process most of the chemical products used remain in the original state; in fact the quantities fixed are almost always lower than those supplied.

For example, in the case of conventional chrome tanning, the fixation efficiency almost never exceeds 70%. This involves on the one hand high consumption and waste of raw materials, on the other the presence of high quantities of chemical products in the discharges with consequent need for purification treatments. In addition, the tanning production cycle, which transforms raw leather into finished leather with a limited yield, also generates a high quantity of waste that can be divided into solid untanned residues (e.g. fleshings) and solid tanned residues (e.g. shaving and leather scraps). In the literature it is reported that from a ton of raw hides, about 200 kg of leather and about 500- 600 kg of solid residues are produced.

In this work we describe the current knowledge on the use of enzymatic technologies in the various processing phases of the tanning process (soaking, unhairing, bating, degreasing, ecc.) for the purpose of reducing the polluting load. Furthermore, taking into account the nature of the main constituents of solid tanning waste (proteins and fats) it is also possible to think about their reuse in a circular economy perspective by means of an adequate enzymatic treatment

Keywords: enzymes , circular economy, tanning industry, biotechnology

[1] B. Naviglio, G. Calvanese, D.Caracciolo, C.Florio, T. Gambicorti, The circular economy in the tanning industry, XXI Congresso Divisione Chimica Industriale – Salerno 27 agosto 2019

FISH chitinolytic biowastes FOR FISH active and sustainable packaging material: the FISH4FISH EU project*

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Every year, 6-8 million of tonnes of waste crab, shrimp and lobster are produced globally. In developing countries, waste shells are often dumped in landfill or the sea. In developed countries, disposal can be costly. Nonetheless shells harbour many chemicals like proteins, pigments, calcium carbonate and chitin. Often the term "Shell biorefinery"[1,2] is used in analogy with "Wood biorefinery". In this context, the FISH4FISH project aims to produce innovative active and sustainable packaging material based on chitinolytic derivatives, using marine biomass wastes. The fish industry has a strong need to improve marketability and to extend the shelf-life of fresh fish, shortened by microbial spoilage. Packaging plays a critical role in the fish supply chain and can be part of the solution to tackle food waste. Packaging based on chitinolytic derivatives is able to tackle microbial spoilage, enhancing fish shelf life, and, in the post-consumption phase it can be used as fertilizer and microbial preservatives for plants. The project will leverage on the enormous biotechnological potential (prebiotic activity, antioxidant and antimicrobial properties) of the chitosan and above all chito-oligosaccharides. Furthermore lignin nanoparticles will be used as active biofiller in the preparation of the new polymeric materials. The ambitious objective of the FISH4FISH project is to obtain a low cost active material to be exploited for the industrial applications of fish packaging. In this way, renewable resources are exploited in a sustainable manner, promoting bio-based, environmentally friendly and beneficial technologies, and create high-performing materials for a wide range of applications.

1. Ning, Y.; Xi, C. *Nature.* **2015**, *524,* 155-157.
2. Hulsey, M.J. *Green Energy & Env.* **2018**, 3, 318-327

*Project co-funded by EU under the program **EASME/EMFF/Blue Economy-2018/n.863697**

3D human tissues within a patented fluidic system (MIVO) to improve the outcome of in vitro assays of pharma, dermo-cosmetic and nutraceutical products

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In vitro reproducible and quantitative studies of molecules absorption through biological interfacing tissues are quite limited due to the lack of reliable experimental models able to resemble the in vivo responses. In this work, we present a novel platform as *in vitro* dynamic model of healthy and pathological tissues integrated with a fluidic bioreactor resembling the *in vivo* systemic stimuli and the organ-organ fluidic connections. The MIVO® -Multi In Vitro Organ - platform has been validated in different applications[1,2], such as: (i) the intestinal permeability of molecules, which is crucial in regulating the bioavailability and consecutively the biological effects of drugs and compounds, (ii) the drug efficacy assay against ovarian cancer tumor, the tumor cell migration and their survival under shear stress induced fluid flow, (iii) the medical device absorption through skin tissues according to ADME regulations.

The results showed that the MIVO® platform combined with human 3D tissues (Figure 1) can represent a novel, reliable and easy-to-use in vitro model of interfacing phenomena for studying the passage of bioactive molecules of drugs and their effect on cellular behavior.

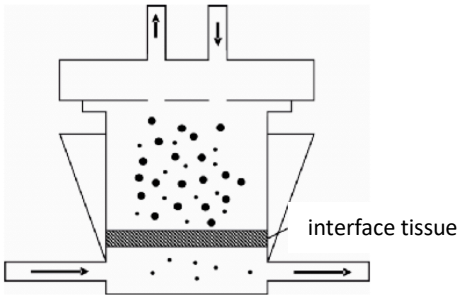

Figure 1: Scheme of the MIVO platform

1.Cavo M., Caria, M.m Pulsoni I. et al. *Sci Rep* **2018** 8:5333
2. Marrella A, Buratti P, Markus J, Firpo G, Pesenti M, Landry T, Ayehunie S. et al. *ALTEX* **2019** Dec 30.

Chemical strategies for the development of innovative hybrid complexes for the diagnosis and treatment of human diseases

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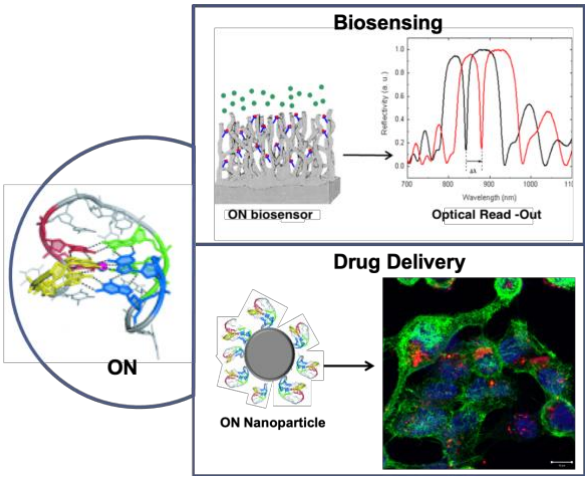
The wide availability of bioactive molecules and new nanomaterials paves the way towards the obtainment of innovative hybrid complexes provided with enhanced efficacy in the biomedical fields compared to their isolated precursors. In this context, the application of hybrid complexes (e.g., bioengineered molecules, nanoparticles or surfaces) for on-demand diagnosis and therapy of human diseases has rapidly become an intriguing research topic. For example, oligonucleotides (ONs) and ON analogues (e.g. modified aptamers or peptide nucleic acids) have been explored as bioprobes (i.e., sensing element) for the realization of label-free biosensors for the early diagnosis of human diseases[1]. The construction of the hybrid devices can be done by coupling the *ex-situ* synthesized bioprobe on the transducer surface, or by direct solid-phase synthesis of the growing bioprobe on the transducer surface (used here as the solid support)[2]. In this communication, I will present the different chemical approaches which we are exploring in our laboratories for the development of label-free hybrid biosensors provided with great stability, fast response time, high sensitivity and specificity to be used for targeted drug delivery applications[3, 4].



Schematic representation of ON and ON analogues used in biosensing and drug delivery applications.

1. N. Borbone, et al. *J. Med. Chem*., **2012**, 55, 10716–10728
2. M. Terracciano, et al. *RSC Advances,* **2016**, 6, 86762–86769
3. F. Zarrilli, et al., *Molecules*, **2017** *22*, 7, 1144.
4. A.P. Falanga, *Bioconjug. Chem.* ***2019***, *30*, 3, 572–582

Self-assembly of gc PNA-diphenylalanine building blocks

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Self-assembly is a process in which components, either separate or linked, spontaneously form ordered aggregates. The use of short peptides or modified nucleotides as building blocks for the aggregates is particularly intriguing for the production of novel materials, electronic nanodevices and biosensors[1]. Molecules containing both a peptide backbone and a nucleobase also showed interesting optoelectronic properties. Previous studies demonstrated that peptide nucleic acid (PNA)-based molecules are able to spontaneously self-assemble into fluorescent fibrillary aggregates[2]. Moreover, fluorenylmethyloxycarbonyl (Fmoc)-protected gc PNA dimer forms aggregate at a very low concentration in organic solvents, to give fluorescent spherical structures characterized by a very high quantum yield in the visible range with a lifetime in the nanosecond range[3]. Finally, recently we also demonstrated that the conjugation of mono or dimer PNAs to the dipeptide Phe–Phe (FF) leads to the formation of novel hybrid assemblies, characterized by an amyloid-like association of the monomers and with promising features for the development of novel optoelectronic materials[4]. Starting from this observation, here we describe the self-assembling properties in water of the aggregates formed by gc PNA dimer conjugated to the FF dipeptide. In order to realize how opportunely modulate the optical properties of the resulting material, we synthetized four different variants, in which the FF moiety is alternatively positioned at the C or at the N-terminus and the C-terminus is amidated or in its carboxylated form. All the peptide derivatives were full characterized from the structural and spectroscopic point of view. As expected, our results indicate that a punctual chemical modification of the building block can deeply affect the functional properties.

1. Davis, J.; T.; Spada, G.; P. *Chem. Soc. Rev.***2007**, *36*, 296 -313.

2. Berger, O.; Gazit, E. *Nat. Nanotechnol.* **2015**, *10*, 353-360.

3. Avitabile, C.; Diaferia, C.; Della Ventura, B.; Mercurio, F.; A.; Leone, M.; Roviello, V.; Saviano, M.; Velotta, R.; Morelli, G.; Accardo, A.; Romanelli, A. *Chem. Eur. J.* **2018**, *24*, 4729–4735.

4. Avitabile, C.; Diaferia, C.; Roviello, V.; Altamura, D.; Giannini, C.; Vitagliano, L.; Accardo′ A.; Romanelli, A. *Chem. Eur. J.* **2019**, *25*, 14850-14857.

CW-Multifrequency and Pulse EPR to discriminate melanin pigments of different composition

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Melanins consist of natural pigments found in most organisms. Melanin is produced through a chemical process called melanogenesis and it is present in different forms: eumelanin, pheomelanin and pyomelanin. The first one is the most common and it is a black/brown pigment which is produced by the enzymatic oxidation of tyrosine through 3,4-dihydrophenylalanine (DOPA), the second one is a cysteine-derivative responsible for the yellow/reddish colour produced by the same oxidation pathway of tyrosine but including reaction with sulphydryl groups of cysteines, while pyomelanin is mainly found in bacterial and fungal sources. During the reaction processes these pigments form stable radicals and they are an interesting field for Electron Paramagnetic Resonance (EPR) technique studies [1]. Melanins are involved in UV-radiation absorber to metal ions chelators, free radical scavengers and ionic-electronic conductors. Therefore, they represent a very interesting field of research due also to the applicability in melanin-inspired biomaterials production [2-4].

In this work, a Multifrequency CW EPR (X-, Q-band) and Pulse EPR at Q-band have been applied to discriminate melanin pigments of different composition. The paramagnetic properties of enzymatically produced dopa-melanin and cysteinyldopa melanin, using selective microwave pulses, were studied in order to measure longitudinal relaxation times. The results provide the evidence that faster relaxation dynamics are present in cystenyldopa melanin and that pulse EPR represents a useful tool to discriminate one contribution to the other [5].

1. Desmet, C.M.; Danhier, P.; Acciardo, S.; Leveque, P.; Gallez, B. *Free Radical Research* **2019**, *53(4),* 405-410.
2. Monster, A.B.; Rienecker, S.B.; Noble, C.; Hanson, G.R.; Meredith, P. *Sci. Adv.* **2018**, *4,* 1-7.
3. Monster, A.B.; Hanson, G.R.; Sarna, I.R.; Gentle, R.; Powell, B.J.; Meredith, P. *J. Phys. Chem. B* **2013**, *117,* 4965-4972.
4. Chen, C.T.; Chuang, C.; Cao, J.; Ball, V.; Ruch, D.; Buehler, M.J.; *Nat. Commun.* **2014**, *5,* 1-10.
5. Al Khatib, M.; Costa, J.; Baratto, M.C.; Basosi, R.; Pogni, R. *J. of Phys. Chem. B* **2019**, *submitted, Manuscript ID: jp-2019-11785b*

MicroNeedles: a painless door for transdermal drug delivery

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Microneedles are microscopic applicators used to deliver vaccines or other drugs through transdermal application. They are a painless alternative to the hypodermic syringe, topical creams and transdermal patches. The effect of most of the therapeutic agents is limited due to the stratum corneum layer of the skin, which serves as a barrier for the molecules and thus only a few molecules are able to reach the site of action. A new form of delivery system called the microneedles helps to enhance the delivery of the drug through this route and overcoming the various problems associated with the conventional formulations.
These devices enable high performance in both drug delivery and diagnostic analysis and are constructed through various methods usually involving photolithographic processes or micromolding.
In our innovative approach, polymeric microneedles are fabricated by direct photolithography, without any etching or molding process. A mixture of polyethylene glycol and a commercial photocatalyzer, is exposed to ultraviolet light (365 nm) through a mask, obtaining MNs of different shape, length, density and tip. Depending on the specific application, physical and chemical characteristics of these devices can be tuned as well as the material of which they are made of. Furthermore, the various types of microneedles can be fabricated like dissolving, hydrogel, coated and hollow microneedles.
Microneedles are applied to a patch. The arrays are, then, applied to the skin of patients and are given time to allow for the effective administration of drugs.

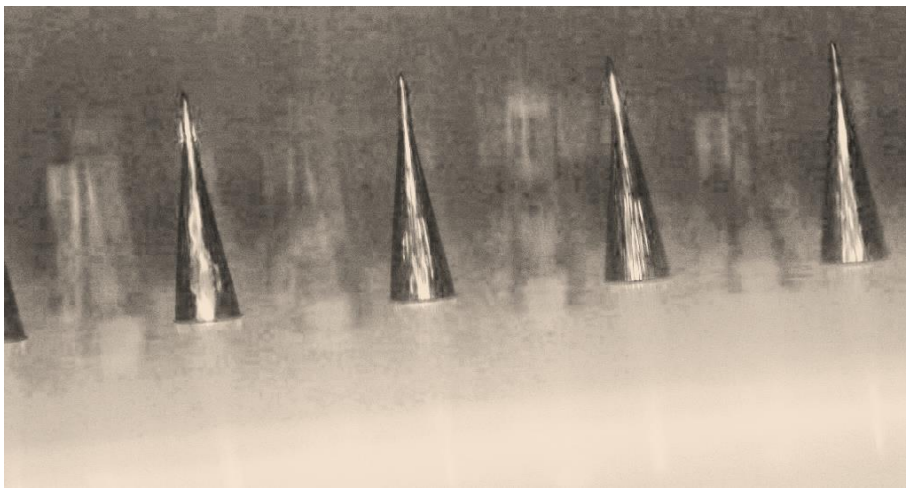

Fig.1

1. Dardano, Principia, et al. "A photolithographic approach to polymeric microneedles array fabrication." *Materials* 8.12 (2015): 8661-8673.

Cheese whey as a source of chemical building blocks by enzymatic hydrolysis of lactose

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Agri-food waste (i.e. waste from agricultural and food industries) are a key towards bioeconomy and circular economy approaches [1]. The dairy industry is one of the most relevant sectors of the Italian food industry and cheese represents its main product. Cheese whey (CW) is the main by-product of the cheese making (Fig.1), and it is currently considered a serious pollutant [2].

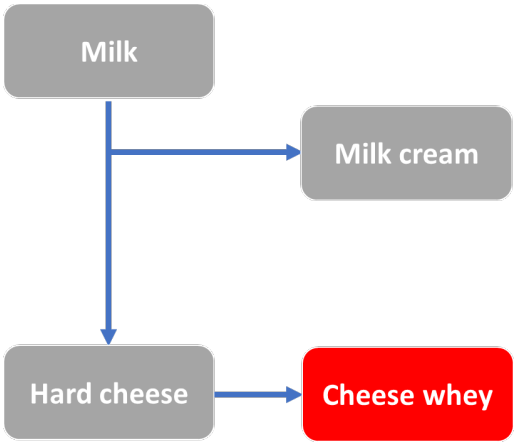

Figure 1. *Products and by-products of cheese industry: usually, 1 kg of cheese is obtained from 10 kg of milk, generating 9 kg of CW*

Whey possesses a high potential to be valuable raw material for added value food, bioactive substances, fermentation feedstock. Lactose represents the main pollutant of CW. Here we proposed the use of a novel Antarctic β-galactosidase (β-Gal_M) for the enzymatic hydrolysis of CW lactose, to obtain glucose and galactose as building blocks for chemical transformations. Easily produced as a recombinant protein, β-Gal_M has proved to be a very stable enzyme, specific for the hydrolysis of β-galactoside bond. Furthermore, the activity in a broad range of temperature (from 5°C to 50°C) makes this enzyme suitable to investigate the exploitation of CW by lactose hydrolysis. According to circular economy principles, this type of process could be advantageous above all when realized in a context where CW, and the lactose it contains, represents an abundant resource, widely accessible but not yet fully exploited. This is the case of the CW produced in Lombardy and its by-products poor in proteins but rich in lactose.

[1] *http://ec.europa.eu/research/bioeconomy*

[2] S. R. Macwan et al. Whey and its utilization. Int.J.Curr.Microbiol.App.Sci **2016**, *5*,134-155.

Comparison of two different synthetic routes of magnetic Fe_3O_4 nanoparticles for enzymatic immobilization

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The use of magnetic nanoparticle represents an important innovation in biotechnology field in enzymatic immobilization process. Their small size increases the contact surface, consequently more enzyme could be immobilized. This technique allows the enzyme to be reused and be easily separated from the reaction environment applying an external magnetic field.

Magnetic nanoparticles (MNPs) were synthetized using two different routes. The alkaline co-precipitation of ferrous and ferric ions was used for both of them but different parameters were changed such as the nature of iron salts, Fe^{2+}/Fe^{3+} molar ration and precipitating agent.[1] The pH stability of MNPs-1 and MNPs-2 was tested monitoring the size and ζ-potential from pH 4 to 9. The size and morphologic characterization of MNPs-1 and MNPs-2 were determined by Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) as well as the chemical structure (FT-IR). Laccase from *Trametes versicolor* (*T.v.*) was immobilized on the magnetic nanoparticles. The MNPs-1 was coated by APTES and glutaraldehyde was used as linker.[2] For MNPs-2, two immobilization methods were carried out: in the first method the laccase was directly adsorbed on nanoparticles, in the second method glutaraldehyde was used to connect the enzyme. The immobilization conditions were optimized testing different concentration of laccase, linker and nanoparticles. Moreover, thermal stability and reusability were analysed.[3] Comparison on the enzymatic immobilization performances on the nanoparticles obtained by the different routes is attempted.

1. De Sousa, M.E.; Fernández Van Raap, M.B.; Rivas, C.P.; Mendoza Zélis, P.; Girardin, P.; Pasquevich, G.A.; Alessandrini, J.L.; Muraca, D. *J. Phys. Chem. C.* **2013**, *117*, 5436-5445.
2. Feng, J.; Yu, S.; Li, J.; Mo, T.; Li. P. *Chem. Eng. J.* **2016**, *286*, 216-222.
3. Fatarella, E.; Spinelli, D.; Ruzzante, M.; Pogni, R. *J. Mol. Catal. B Enzym.* **2014**, *102*, 41-47.

Targeting Axl receptor in drug discovery

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Axl is a tyrosine kinase receptor playing key role in several biological processes, as its activation promotes cell proliferation, survival, migration and angiogenesis.[1] Axl receptor exhibits a cytoplasmic TK domain, a transmembrane region and an extracellular portion, harboring two N-terminal immunoglobulin (Ig)-like domains and two fibronectin type III (FNIII) repeats. The growth arrest specific protein-6 (Gas6) is the natural ligand of Axl.[2] Gas6 binds to the extracellular portion of Axl, leading to receptor dimerization and activation. The deregulation of Axl signaling has been associated to several high impact diseases, such as cancer and multiple sclerosis.[3,4] Thus, Axl is emerging as a novel and interesting molecular target in drug discovery. Molecules that selectively binds to Axl receptor appear useful in biomedicine to develop new therapeutic agents or diagnostic tools. We intend to develop novel peptide molecules targeting Axl receptor using the phage display library screening technique. To this aim, we prepared by recombinant means the two extracellular fibronectin type III domains (FNIII-1 and FN-III-2) of Axl to be used as bait in phage display experiments. We also set-up the synthetic procedure for the synthesis by native chemical ligation of FNIII-1 and FN-III-2 in their D-enantio form, which allow the selection of metabolically stable D-peptide binders of Axl using the innovative screening technique called "mirror image phage display".[5]

1. Linger, R.M. et al. *Adv. Cancer Res.* **2008**, 100, 35-83.
2. Varnum, B.C. et al. *Nature.* **1995**, 373(6515), 623-626.
3. Gay, C.M. et al. *Br. J. Cancer.* **2017**, 116(4), 415-423.
4. Bellan, M. et al. *Int J Mol Sci.* **2016**, 17(11), pii: E1807.
5. Schumacher, T.N. et al. *Science.* **1996**, 271(5257), 1854-1857.

Development of an innovative biosensor for diagnosing Chronic Lymphocytic Leukemia: a PNA probe can detect CD5 biomarker in vitro.

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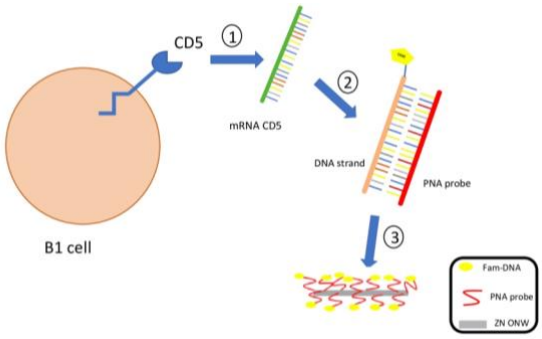
A growing demand for small, fast, efficient and portable devices able to detect a specific analyte has meant biosensing one of the most rapidly expanding research field, ranging from a broad of applications including clinical diagnostics, environmental monitoring and food quality control. A biosensor is a hybrid device constituted by a biomolecular probe, employed to selectively recognize a biochemical target, immobilized on a transducer surface which converts the target recognition into an analytical signal. In this work we proposed the realization of an innovative biosensor for diagnosing Chronic Lymphocytic Leukemia by using a Zinc Oxide Nanowires (ZN ONWs) as transducer surface and PNA as bioprobe able to recognize diagnostic marker CD5 mRNA (scheme 1). At first, we identified a short region of the transcript to employ as template for the synthesis of the PNA[1]. Circular Dichroism (CD), CD Melting and Non-Denaturing Polyacrylamide Gel Electrophoresis (PAGE) were performed to investigate the capability of PNA to recognize the complementary DNA, both in presence and in absence of a fluorescent residue (FAM). All experiments demonstrated the formation of a stable heteroduplex PNA-DNA. Then. we proceeded with the realization of the PNA-biosensor. In particular, chemical strategy based on silane (*e.g.* APTES) was used as treatment to passivate and functionalize the trasducer surface with the bioprobe[2-4]. The ability of the device to hybridize the target sequence was analyzed by electrical and optical measurements. In conclusion, the results assessed that the PNA functionalized biosensor is able to form a stable complex only with the complementary DNA strand compared to the negative control of DNA.



Scheme 1. Schematic representation of the procedure

Reference

1. Faguet GB et al. Leukemia & Lymphoma 1992, 6, 335-344.
2. Terracciano et al. *RSC Advances* 6 (2016) 86762-86769.
3. R. Moretta et al. *Frontiers in Chemistry* 6 (2018) 583.
4. L. De Stefano et al. *Journal of the Royal Society Interface* 10 (2013) 20130160.

Orthogonal bio-chemiluminescent paper-based biosensor for acetylcholinesterase activity detection

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Global security threats have become a major worldwide concern and their early and sensitive detection represents a major challenge to current detection technologies. The routine monitoring of water, food and the environment for chemical and biological threat agents is often hampered by the fact that available techniques usually require clean samples and sophisticated equipment unsuitable for real-time, cost-effective and on-field routine monitoring. The possibility of implementing enzymatic assays with bio-chemiluminescence detection in smartphones has been very successful over the years, as well as the possibility of creating ad hoc analytical devices manufactured with an easy and economical 3D printing technology. Here, we report the development and optimization of an orthogonal paper-based bio-chemiluminescent (BL-CL) biosensor for acetylcholinesterase activity detection and its implementation into portable analytical devices as proof-of-principle of low-cost point-of-care applications. This biosensor is based on the inhibition process of acetylcholinesterase (AChE) by molecules such as organophosphate pesticides, nerve gases and some drugs. As regards the chemiluminescent reaction, AchE activity is measured through a series of coupled enzymatic reactions (exploiting AChE, choline oxidase (ChOx) and Horse Radish Peroxidase (HRP)) leading to light emission. When acetylcholinesterase is inhibited, there is a decreased production of hydrogen peroxide, and consequently a reduction in light emission. Regarding the bioluminescent reaction, the first reaction is catalyzed by acetylcholinesterase, generating acetate and choline. Acetate, the product of this first reaction, is one of the substrates for S-acetyl-coenzyme A synthetase, along with ATP and coenzyme A. The final coupled reaction is catalyzed by firefly luciferase, requiring the substrate firefly luciferin and ATP. However, because ATP is consumed in the previous reaction, the bioluminescent reaction is impaired. The presence of an organophosphorus pesticides will inhibit the first reaction, thus favouring the bioluminescent reaction. The origami technique allows to add reagents in separate steps and trigger the reactions to occur sequentially. Signal acquisition was carried out by OnePlus 6 photocamera placing the 3D paper PAD cartridge inside a 3D-printed dark box and integrating BL-CL signals for 30 sec with ISO800.

Multiparametric Porous Silicon Optical Biosensor for Brugada Syndrome Diagnosis

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Brugada syndrome is a genetic disorder with autosomal dominant transmission, associated to sudden death in young patients with a structurally healthy heart. As this pathology is difficult to diagnose, a multiparametric hybrid graphene oxide - porous silicon biosensor has been developed for tempestive diagnosis of this disease. Graphene oxide is a sp^2 carbon-based layered material, characterized by the oxygen functional groups present on the whole surface (i.e., carboxyl, epoxy and hydroxyl groups) that ensure the water-solubility of the material and they serve as site for chemical functionalization [1], [2]. Moreover, GO shows a broad PL emission from 500 to 800 nm, which was proposed for the development of a new class of optoelectronic devices [3]. Unfortunately, the PL emission of GO is weak, due to the oxygen-functional groups producing non-radiative recombination between their electrons and holes present in sp^2 clusters. A valid approach to enhance the GO PL is the infiltration of the material into large specific area substrate, such as PSi. PSi is a nanostructured material, obtained by electrochemical dissolution of doped crystalline silicon. The sponge-like morphology, characterized by a high surface area, make PSi a transducer material ideal for biosensing applications [4]. In recent papers, amino-modified monolayer and multi-layered PSi structures were used to infiltrate GO nanosheets by spin-coating. The enhancement and the modulation of the PL signal emitted from GO adsorbed on the hybrid structures were highlighted, while this effect was not evident in the case of GO on crystalline flat silicon [5], [6]. Here, a covalent approach is proposed to immobilize the GO on PSi surface. A peptide nucleic acid (PNA) sequence is used as bioprobe, covalently linked to the device, able to recognize the DNA mutation associated to Brugada Syndrome. The device is an optical biosensor whose operating mechanism is based on the changes of PSi reflectance and GO photoluminescence.

[1] D. R. Dreyer, S. Park, C. W. Bielawski, and R. S. Ruoff, *Chem. Soc. Rev.*, vol. 39, no. 1, pp. 228–40, Jan. 2010.

[2] M. Wu, R. Kempaiah, P.-J. J. Huang, V. Maheshwari, and J. Liu, *Langmuir*, vol. 27, no. 6, pp. 2731–8, Mar. 2011.

[3] C.-T. Chien *et al.*, *Angew. Chemie Int. Ed.*, vol. 51, no. 27, pp. 6662–6666, Jul. 2012.

[4] M. J. Sailor, Wiley-VCH, 2012.

[5] I. Rea *et al.*, *J. Phys. Chem. C*, vol. 118, no. 47, pp. 27301–27307, Nov. 2014.

[6] I. Rea, M. Casalino, M. Terracciano, L. Sansone, J. Politi, and L. De Stefano, *Opt. Express*, vol. 24, no. 21, pp. 24413–24421, Oct. 2016.

Natural polyphenols and synthetic analogues as digestive enzymes inhibitors: potential antidiabetic and antiobesity agents

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Obesity is a metabolic disorder resulting from an excessive accumulation of body fat. It is associated with a myriad of comorbidities, such as the onset of cardiovascular diseases, diverse types of cancer, osteoarthritis, and hypertension.[1] Moreover, the incidence of obesity is frequently associated with the incidence of type 2 diabetes (Diabetes mellitus), a metabolic disorder characterized by insulin hormone dysfunction, and as a result, by high blood glucose levels. Type 2 diabetes represents 90% of all diabetes cases and among other diet-related diseases is the primary cause of deaths. It is also noteworthy that hyperglycemia associated with Diabetes mellitus is characterized by an increases the production of reactive oxygen species, causing oxidative tissue damage.

Several strategies have been developed for the inhibition of the enzymes involved in the dietary disease.

Pancreatic lipase is a key enzyme in dietary fat absorption, responsible for the hydrolysis of 50–70% of dietary triglycerides into monoacylglycerides and free fatty acids, which can then be absorbed by enterocytes. Inhibition of this enzyme is used to reduce dietary fat absorption. α-Amylase and α-glucosidase are carbohydrate hydrolyzing enzymes, and their inhibition is currently one of the strategies to manage the hyperglycemia resulting from T2D. Several drugs are currently employed as inhibitors of α-amylase, α-glucosidase and pancreatic lipase although with several undesired side effects.

In recent years, some natural polyphenols have been reported as inhibitors of the above digestive enzymes, involved in metabolic diseases. The present work reports some of our recent efforts aimed at discovering the inhibition properties of natural polyphenols or synthetic analogues. [2-4] Several examples will be provided : 1) dimeric neolignans and their synthetic analogues 2) isoflavonoids 3) C-glucosidic ellagitannins and galloylated glucoses.

[1] WHO. World Health Organization; 2015. Obesity and Overweight. http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed January 2020)
[2] Pulvirenti, L.; Muccilli, V.; Cardullo, N.; Spatafora, C.; Tringali, C. J. Nat. Prod. **2017**, *80*, 1648−165
[3] Cardullo, N.; Catinella, G.; Floresta, G.; Muccilli, V.; Rosselli, S.; Rescifina, A..; Bruno,† M.; Tringali, C. J. Nat. Prod. **2019**, *82*, 573−582
[4] Cardullo, N.; Muccilli, V.; Pulvirenti, L.; Cornu, A.; Pouységu, L.; Deffieux, D.; Quideau, S.; Tringali C. Food Chem. **2020**, *313* 126099

An efficient immobilized ω-transaminase (TA-IMB) for the preparation of some industrially relevant amines

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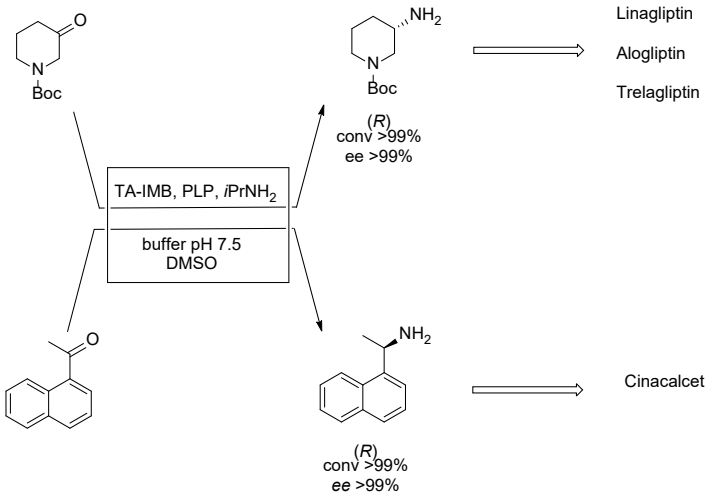
The use of ω-transaminases (TAs) has been identified as a greener tool for the preparation of enantiomerically pure amines which are important reagents or intermediates of biologically active industrial compounds.[1] Frequently these commercial enzymes are used in free form but, in order to obtain a process intensification and potential reduction of costs, immobilized TAs would be preferable. As a matter of fact, immobilized biocatalysts may be more stable to higher temperatures than free enzymes and less susceptible to the inactivation from solvents, reagents and products. Furthermore, they may be re-used in batch and in continuous-mode reactions and reaction work-up and purification of the target product are simplified. For an economic evaluation however it is necessary to consider that the activity of an immobilized enzyme is lower than free enzyme and its cost is comparable only if more recycles are possible. In this context a procedure was developed on lab scale to evaluate feasibility and potential costs of the synthesis on a larger scale of the (R)-3-amino-1-Boc-piperidine and (R)-1-(1-naphtyl)ethylamine (Scheme), which are respectively key chiral amine reagents in the preparation of some antidiabetic drugs, such as Linagliptin, Alogliptin and Trelagliptin, and of Cinacalcet, a calcimimetic agent. An efficient commercial TA-IMB enzyme (ZAM14151-1), which is potentially cheaper on industrial scale than other commercial sources previously checked by us,[2] was used. Preliminary results with a free TAs but stabilized in a deep eutectic solvent (DES) will be also presented for a comparison.



Scheme

[1] Kelly, S. A.; Pohle, S.; Wharry, S.; Mix, S.; Allen, C. C. R.; Moody, T. S.; Gilmore, B. F. *Chem. Rev.* 2018, **118**, 349-367.
[2] Petri, A.; Colonna, V.; Piccolo, O. *Beilstein J. Org. Chem.* 2019, 15, 60-66.

3D-Chitosan structures loaded with gallic acid: a good candidate for agrifood and pharmaceutical applications

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Chitosan (CS) is a cationic linear polysaccharide consisting of D-glucosamine linked with N-acetyl D-glucosamine residues by β-1-4-linkages. It can be obtained by the deacetylation of chitin, a polymer found in the exoskeleton of crustaceans and insects. Due to its biodegradability, biocompatibility, non-toxicity, and low immunogenicity, chitosan is considered a very promising eco-friendly biopolymer for different purposes. In fact, it has prospective applications in many and different fields including biomedicine, waste waters treatment, cosmetic, pharmaceutic, and agrifood. Moreover, chitosan-based materials can be designated in different forms, as gels, films and porous scaffolds. However, the special macromolecular structure of chitosan results in insufficient biological activities and scarce antioxidant capacity, so limiting its practical applications. To overcome these drawbacks, a phenolic acid (gallic acid, GA) has been introduced into the chitosan structure by a simple adsorption process. The aim of the present study was to obtain and characterize 3D-Chitosan structures loaded with GA for potential applications both in the agrifood and pharmaceutical field. 3D chitosan structures were manufactured integrating different methodological approaches (i.e. preparation of the polymer-solvent solution, freeze-drying). The process and solution parameters were properly optimized to ensure the integrity and reproducibility of the structures.

The chemical (^{1}H-NMR and UV- vis spectra), morphological (Scanning Electron Microscopy - SEM, and Transmission Electron Microscopy - TEM) and biological (antioxidant activity) analyses of the samples obtained showed that 3D-chitosan structures loaded with gallic acid are good candidates for agrifood and pharmaceutical applications.

Delivery of peptide nucleic acids using guanidiniumcalix[4]arenes as vectors

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Despite their relevant potentiality, the application of Peptide Nucleic Acids (PNAs),[1] mimics of DNA lacking the sugar-phosphate backbone, for antisense[2]/anti-gene[3] therapy and gene editing[4] is strongly limited because of their low uptake by cells.[5] Currently, no simple and efficient delivery systems and methods are available to solve this open issue. One of the most promising approach is the modification of the PNA structure through the covalent linkage of poliarginine tails,[6] but this means that every PNA intended to be internalized must be modified. Herein we report the results relative to the delivery ability of macrocyclic multivalent guanidiniumcalix[4]arenes[7] used as non-covalent vector for anti-miRNA PNAs.[8] These non-viral delivery systems are characterized by amphiphilicity which results fundamental for the transfection activity as much as the presence of the guanidinium units as polar cationic heads. High delivery efficiency, low cytotoxicity, maintenance of the PNA biological activity and ease preparation of the transfection formulation, simply attained by mixing PNA and calixarene derivative, candidate these vectors as universal delivery system for this class of nucleic acid analogues.

1. Nielsen, P. E.; Egholm, M.; Buchardt, O. *Bioconjugate Chem.* **1994**, *5*, 3–7.
2. Larsen, H. J.; Bentin, T.; Nielsen, P. E. *Biochim. Biophys. Acta* **1999**, *1489*, 159–166.
3. Nielsen, P. E. *Curr. Pharm. Des.* **2010**, *16*, 3118–3123.
4. Ricciardi, A. S.; Quijano, E.; Putman, R.; Saltzman, W. M.; Glazer, P. M. *Molecules* **2018**, *23*, 632.
5. Bertucci, A.; Prasetyanto, E. A.; Septiadi, D.; Manicardi, A.; Brognara, E.; Gambari, R.; Corradini, R.; De Cola, L. *Small* **2015**, *11*, 5687–5695.
6. Brognara, E.; Fabbri, E.; Montagner, G.; Gasparello, J.; Manicardi, A.; Corradini, R.; Bianchi, N.; Finotti, A.; Breveglieri, G.; Borgatti, M.; Lampronti, I.; Milani, R.; Dechecchi, M.; Cabrini, G.; Gambari, R. *Int. J. Oncol.* **2016**, *48*, 1029–1038.
7. Bagnacani, V.; Franceschi, V.; Bassi, M.; Lomazzi, M.; Donofrio, G.; Sansone, F.; Casnati, A.; Ungaro, R. *Nat. Commun.* **2013**, *4*, 1721.
8. Gasparello, J.; Manicardi, A.; Casnati, A.; Corradini, R.; Gambari, R.; Finotti, A.; Sansone, F. *Sci. Rep.* **2019**, *9*:3036.

At the interface between Chemistry and Biology: targeting RNA with dipeptidyl-anthraquinone conjugates to develop new potential anti-HIV-1 agents

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In this multidisciplinary study we analyze a set of compounds with potential significance to the therapy of viral infections thanks to their inhibitory activity of two HIV-1 proteins, namely the nucleocapsid (NC) protein and the trans-activator of transcription (Tat) peptide. The evaluated molecules belong to our chemical library[1-3] obtained by conjugating a scaffold highly represented in natural products, such as the anthraquinone core, with small peptides with nucleic acid binding properties. We demonstrate that properly substituted dipeptidyl-anthraquinone conjugates efficiently inhibit the NC- and Tat-mediated RNA chaperone activity in reverse transcription process, and that such inhibition is related to the RNA binding properties of the hit compounds as demonstrated by high resolution mass spectrometry. Dual targeting through the same RNA regulatory sequences opens the possibility to consider dipeptidyl-anthraquinone conjugates as pleiotropic inhibitors, demonstrating the possibility to develop multi-target compounds capable of interfering with processes mediated by the interactions of this essential RNA domain of HIV-1 genome with viral proteins.

1. Sosic A. et al, *Med. Chem. Commun.*, **2013**, 4, 1388–1393.
2. Frecentese F., Sosic A. et al, *J. Med. Chem.*, **2016**, 59, 1914−1924.
3. Sosic A. et al, *Bioconjugate Chem.*, **2018**, 29, 7, 2195-2207