Società Chimica Italiana
Gruppo Interdivisionale
Biotecnologie

5° Workshop

I chimici per le biotecnologie

Lunedì 27 Febbraio 2023 – Ore 9.30-17.00
***Aula T8 – Centro CESTEV** (Palazzo delle Biotecnologie)*
Via Tommaso De Amicis, 95
Università degli Studi di Napoli Federico II

Sponsorizzato da:

Col patrocinio di:

Università degli Studi di Napoli
FEDERICO II
Dipartimento di Medicina Molecolare e
Biotecnologie Mediche

Società Chimica Italiana
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PROGRAMMA

9:00	9:30	**Accoglienza partecipanti**		
		Sessione mattutina (in italiano)		
9:30	10:00	**Apertura lavori e saluti di benvenuto** Prof.ssa Giorgia Oliviero – Coordinatrice GIB Prof.ssa Maria Valeria D'Auria – Ex presidente della Divisione di Chimica Organica della SCI Prof. Gennaro Piccialli – Direttore CESTEV		
10:00	10:25	**Invited talk**		Chairs: Prof.ssa Serena Riela/Prof. Alessandro Palmioli
		IT1 **Monica Notarbartolo di Villarosa**	Università di Palermo	*Clay minerals nanomaterial-based vectors for biological applications*
10:25	11:10	**Flash Communications**		
10:25	10:30	**FC1** **Roberto Nilo**	CNR - IEOS	*Development of novel aptamer-guided nanoparticles for specific triple-negative breast cancer treatment*
10:30	10:35	**FC2** **Gianluca Ciarleglio**	Università di Roma	*Multi-responsive microspheres for the effective delivery of OZOILE®*
10:35	10:40	**FC3** **Daniela Benigno**	Università di Napoli Federico II	*New G-quadruplex DNA catalysts in enantioselective sulfoxidation reaction*
10:40	10:45	**FC4** **Federico Zappaterra**	Università di Ferrara	*Biocatalytic Synthesis of an ibuprofen prodrug: xylitol as hydrophilization moiety*
10:45	10:50	**FC5** **Simona Aprile**	Università di Ferrara	*Enzymatic synthesis of ascorbic acid-ketone bodies hybrids*
10:50	10:55	**FC6** **Luca Pisano**	Università di Roma	*Ferritin chemical modification for the multifunctional nucleic acid delivery*
10:55	11:00	**FC7** **Francesca Cardano**	Università di Torino	*Intrinsically emissive peptide nucleic acids*
11:00	11:05	**FC8** **Agostina Colacicco**	Università di Milano	*Flow biocatalyzed reactions for the preparation of valuable aglycones from the corresponding natural glycosides*
11:05	11:10	**FC9** **Aniello Stellato**	**T.M.E.**	*Global strategy: Mission-Vision*
11:10	11:30	**Coffee Break**		

11:30	11:55	**Invited talk**		Chairs: Prof.ssa Maria Paola Costi/Francesco Presini
		IT2 **Riccardo Palmisano**	Ex Presidente Assobiotech	*Lo sviluppo delle Biotecnologie nelle Scienze della Vita in Italia*
11:55	12:10	**Short Communication**		
		SC1 **Brigida D'Abrosca**	Università della Campania, Luigi Vanvitelli	*Plants from the Myrtaceae family as promising sources for biological applications: bio-guided isolation of alkylphloroglucinol glucosides*
12:10	12:55	**Flash Communications**		
12:10	12:15	**FC10** **Emanuela Esposito**	CNR – ISASI	*ISASI-CNR Naples Cryo Electron Microscopy laboratory*
12:15	12:20	**FC11** **Giulia Malpezzi**	Università di Modena e Reggio Emilia	*Exploiting the Optical Tweezer technology to reveal thymidylate synthase interactions with its consensus mRNA*
12:20	12:25	**FC12** **Marina Massaro**	Università di Palermo	*Modification of halloysite lumen with dopamine derivatives as filler for antibiofilm coating*
12:25	12:30	**FC13** **Barbara Prandi**	Università di Parma	*Proteolytic enzymes as a powerful tool for the recovery and biocatalytic conversion of agri-food*
12:30	12:35	**FC14** **Diego Tesauro**	Università di Napoli, Federico II	*Mass spectrometry study of Thioredoxin System targeted by N-Heterocyclic Carbene (NHC)Gold(I) complexes*
12:35	12:40	**FC15** **Lorenzo Tagliazucchi**	Università di Modena e Reggio Emilia	*LC-MS/MS proteomics for the rapid and selective screening of drug resistances in Leishmania infantum clinical isolates*
12:40	12:45	**FC16** **Giovanni Pecoraro**	Synlab SDN	*Organs-on-chip as new frontiers for disease modelling and personalized medicine*
12:45	12:50	**FC17** **Marco Chino**	Università di Napoli, Federico II	*Handcrafted Metal Enzymes Poised for Biotechnology Revolution*
12:50	12:55	**FC18** **Antonella Carillo**	Associazione Biotecnologi Italiani	*Biotecnologi Italiani, da 22 anni con lo sguardo rivolto al futuro*
12:55	13:10	**Short Communication**		
		SC2 **Stefano Volpi**	Università di Parma	*Cationic calix[4]arenes as tools for the intracellular delivery of microRNA mimics and antimiR peptide nucleic acids*

Start	End	Code / Speaker	Affiliation	Title
13:10	13:45	**Flash Communications**		
13:10	13:15	**FC19 Stefania Patti**	Università di Milano	*Insight into the stereoselective synthesis of (1S)-nor(pseudo)ephedrine analogues by biocatalytic cascades*
13:15	13:20	**FC20 Maria Maddalena Calabretta**	Università di Bologna	*Bioluminescence Sensing in 3D Spherical Microtissues for Multiple Bioactivity Analysis of Environmental Samples*
13:20	13:25	**FC21 Rosa Bellavita**	Università di Napoli, Federico II	*Hydroxamate-based peptides as iron(III) chelators for antimicrobial applications*
13:25	13:30	**FC22 Viviana Di Matteo**	Università di Parma	*Stereostructural determination of a new cyanochelin from the cyanobacteria leptolyngbya*
13:30	13:35	**FC23 Karishma Kundu**	Università di Napoli, Federico II	*A Four-Step Platform to Optimize Growth Conditions for High-Yield Production of Siderophores in Cyanobacteria*
13:35	13:40	**FC24 Teresa De Rosa**	Università di Napoli, Federico II	*The biosynthetic potential of cyanobacteria*
13:40	13:45	**FC25 Luca Nespoli**	Università di Napoli, Federico II	*Biocatalytic preparation of tryptamine derivatives as novel antimicrobial agents*
13:45	15:15	**Light lunch Sfogliatella and Poster session**		
		Sessione pomeridiana (in inglese)		
15:15	15:40		**Invited talk**	Chairs: Prof.ssa Laura Cipolla/Prof. Oreste Piccolo
		IT3 Marc Hayes	Enzymaster	*Applying enzyme engineering for commercial manufacturing of chemicals*
15:40	15:55	**Short Communication**		
		SC3 Lucia Lombardi	Imperial College London	*Differential sensing with arrays of de novo designed peptide assemblies*
15:55	16:20		**Invited talk**	Chairs: Prof.ssa Lucia Panzanella/Prof. Alessandro D'Urso
		IT4 Enrico Gallo	Synlab	*Self-assembled peptide-based nanostructures as multivalent tools for biotechnological applications*

16:20	16:50	**Short Communications**		
16:20	16:35	**SC4** **Giulia Romagnoli**	Università di Siena	*Development of new Antibody Drug Conjugates with antiviral activity*
16:35	**16:50**	**SC5** **Maria Letizia Contente**	Università di Milano	*Mycobacterium smegmatis acyltransferase: the big new player in biocatalysis*
16:50	17:15	**Invited talk**		
IT5 **Simas Rackauskas**			Kaunas University of Technology	*ZnO nanowire synthesis and biotechnological application*
17:15	17:25	**Best Poster Award**	Chairs: Prof.ssa Maria Valeria D'Auria/Prof. Francesco Sansone	
17:30		**Chiusura lavori**		

Clay minerals nanomaterial-based vectors for biological applications

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Clay minerals are a well-known class of compounds which have been used for pharmacological applications since ancient times.[1] The medicinal use of clay minerals became more and more prominent when, during the Renaissance, the Pharmacopoeia classified clay minerals as drug. Up to now, clay minerals are widely employed in the pharmaceutical industry as common additives. They are for example used as oral treatment of diarrhea or as gastrointestinal protectors, or for topical dermatological applications.[2]

Among the different clay minerals, the most commonly used ones for pharmaceutical applications are, as example, kaolinite (Kaol), halloysite (Hal), montmorillonite (MMT), hectorite (Ht), and so on, because of their high biocompatibility, availability in large amount at low cost and ability to penetrate in the cell membrane.

This communication will be focused on the latest research on clay minerals for biological applications. In particular, it will be shown the use of halloysite and hectorite as drug carrier and delivery system both for genetic material and chemotherapeutic drugs.

The possibility to develop fluorescent nanoprobes based on clays, that represents a challenge to detect any cellular process under a microscope, will be also discussed.[3-6]

REFERENCES:

1. Carretero, M.I. *Appl. Clay Sci.* **2002**, *21*, 155–163.
2. Massaro, M.; Riela, S. *J. Funct. Biomater.* **2018**, *9*, 58.
3. Notarbartolo, M.; Massaro, M.; de Melo Barbosa, R.; Emili, C.; Liotta, L. F.; Poma, P.; Raymo, F. M.; Sànchez-Espejo, R.; Vago, R.; Viseras-Iborra, C.; Riela, S. *Coll. Surf. B* **2022**, *220*, 112931.
4. Massaro, M.; Licandro, E.; Cauteruccio, S.; Lazzara, G.; Liotta, L. F.; Notarbartolo, M.; Raymo, F. M.; Sánchez-Espejo, R.; Viseras-Iborra, C.; Riela, S. *J. Coll. Int. Sci* **2022**, *620*, 221-233.
5. Massaro, M.; Notarbartolo, M.; Raymo, F.; Cavallaro, G.; Lazzara, G.; Mazza, M. M. A.; Viseras-Iborra, C.; Riela, S. *ACS Appl. Nano Mater.* **2022**, *5*, 13729−13736.
6. Massaro, M.; Poma, P.; Cavallaro, G.; Garcìa-Villén, F.; Lazzara, G.; Notarbartolo, M.; Muratore, N.; Sànchez-Espejo, R.; Viseras-Iborra, C.; Riela, S. *Coll. Surf. B* **2022**, *213*, 112385.

Development of novel aptamer-guided nanoparticles for specific triple-negative breast cancer treatment

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Triple-Negative Breast Cancer (TNBC) is the most heterogeneous and aggressive subtype of breast cancers[1]. Currently, the mainly exploited weapon to treat TNBC is based on chemotherapy[2,3], albeit it possesses numerous disadvantages, such as severe systemic side effects, drug resistance and poor bioavailability[4]. Thus, it is essential to develop novel targeted treatment approaches for TNBC.

Cell-targeting aptamers are single-stranded oligonucleotides able to fold into a tridimensional structure, which allows them to bind specific cell-surface protein at high affinity.[5,6] Thus, they are emerging as highly selective molecules for active cancer cell targeting. Additionally, their conjugation to different types of nanoparticles (NPs) loaded with drugs could produce efficient delivery agents to carry therapeutics specifically into target cells[7]. Our research group has recently selected a panel of nuclease-resistant 2'-fluoro-pyrimidine RNA aptamers able to recognize their specific target protein expressed on the surface of TNBC cells and discriminate them from non-TNBC breast cancer cells[8-12]. These aptamers are able to deliver polymeric nanoparticles loaded with cisplatin[10] or therapeutic anti PD-L1 siRNA[13] to TNBC.

Herein, we report on the synthesis and characterization of two other types of NPs that we are evaluating as platforms for aptamer-conjugation and drug-loading. The first nanosystem, generated in collaboration with the Department of Industrial Chemistry of University of Bologna, consists of an Alginate-derived core, linked to red-emissive carbon dots (RCDs Alg2steps), and functionalized with sTN58 aptamer (sTN58-RCDs Alg2steps). The second nanosystem, generated in collaboration with the CNR NANOTEC Institute, Cosenza, is composed of a gold core and a silica shell, with photosensitive ability, thanks to the water-soluble iridium (III) complex (Ir) inserted into NPs (Ir-AuSiO_2)[14]. The anti-EGFR CL4[10,12] and anti-PDGFRβ Gint4.T[12,15] NH_2-terminated aptamers have been conjugated on Ir-AuSiO_2 surfaces generating Ir-AuSiO2_CL4 and Ir-AuSiO_2_Gint4.T, respectively. Both nanosystems show efficient and specific internalization in human TNBC cell lines, as assessed by confocal microscopy. NPs unconjugated or conjugated with a scrambled aptamer have been used as negative controls.
Concerning the efficacy testing of aptamer-conjugated NPs, MDA-MB-231 cells have been treated with Ir-AuSiO_2_CL4 and Ir-AuSiO_2_Gint4.T, at a concentration up to 5 μM of Ir for 1 h at 37°C, following by washes, 1 h of UV light and 24 h of recovery. A dose-dependent cell mortality up to 70% has been observed in comparison with cells mock-treated or treated with untargeted NPs. The same treatment in dark conditions has not led to a significant rate of mortality. Regarding the alginate-based nanosystems, we are currently evaluating the efficacy of sTN58-RCDs Alg2steps loaded with doxorubicin on TNBC cells.

REFERENCES:

1. Derakhshan F.; Reis-Filho JS. *Annu. Rev. Pathol.* **2022**, 17:181-204.
2. Park JH.; Ahn JH.; Kim SB. *ESMO Open*. **2018**, 3(Suppl 1):e000357.

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3. Li, S.; Bao, C.; Huang, L.; Wei, JF. *J. Clin. Med.* **2022**, 11, 6021.
4. Mross K.; Kratz F. *Drug delivery in oncology*. **2011,** p. 1–31.
5. Camorani S.; Crescenzi E.; Fedele M.; Cerchia L. *Biochim Biophys Acta Rev Cancer*, **2018**, 1869(2):263-277.
6. Shigdar S.; Agnello L.; Fedele M.; Camorani S.; Cerchia L. *Pharmaceutics*. **2021**, 14(1):28.
7. Kim M.; Kim DM.; Kim KS.; Jung W.; Kim DE. *Molecules*. **2018**, 23(4):830.
8. Camorani S.; Granata I.; Collina F.; Leonetti F.; Cantile M.; Botti G.; Fedele M.; Guarracino MR.; Cerchia L. *iScience*. **2020**, 23(4):100979.
9. Camorani S.; d'Argenio A.; Agnello L.; Nilo R.; Zannetti A.; Ibarra LE.; Fedele M.; Cerchia L. *Int. J. Mol. Sci*. **2022**, 23(7):3511.
10. Agnello L.; Tortorella S.; d'Argenio A.; Carbone C.; Camorani S.; Locatelli E.; Auletta L.; Sorrentino D.; Fedele M.; Zannetti A.; Franchini MC.; Cerchia L. *J. Exp. Clin. Cancer Res*. **2021**, 40(1):239.
11. Camorani S.; Crescenzi E.; Gramanzini M.; Fedele M.; Zannetti A.; Cerchia L. *Sci. Rep.* **2017**, 7:46659.
12. Camorani S.; Hill BS.; Collina F.; Gargiulo S.; Napolitano M.; Cantile M.; Di Bonito M.; Botti G.; Fedele M.; Zannetti A.; Cerchia L. *Theranostics*. **2018**, 8(18):5178-5199.
13. Camorani S.; Tortorella S.; Agnello L.; Spanu C.; d'Argenio A.; Nilo R.; Zannetti A.; Locatelli E.; Fedele M.; Comes Franchini M.; Cerchia L. *Pharmaceutics*. **2022**, 14(10):2225.
14. Ricciardi L.; Sancey l.; Palermo G.; Termine R.; De Luca A.; Szerb E.I.; Aiello I.; Ghedini M.; Strangi G.; La Deda M. *Nanoscale*. **2017**, 9, 19279.
15. Monaco I.; Camorani S.; Colecchia D.; Locatelli E.; Calandro P.; Oudin A.; Niclou S.; Arra C.; Chiariello M.; Cerchia L.; Comes Franchini M. *J. Med. Chem*. **2017**, 60(10):4510-4516.

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Multi-responsive microspheres for the effective delivery of OZOILE®

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The increased consumer demand for natural body care products has driven academic and industrial research towards the development of innovative products from raw materials made available by nature. In this perspective, Erbagil srl (Telese Terme, BN, Italy) has developed innovative medical devices based on OZOILE®. OZOILE® is a pool of molecules obtained by a patented ozonation process, in which ozone binds to the olefinic bonds of extra virgin olive oil +Oil® forming stable ozonides. In this process, ozone is produced from medical oxygen and olive oil obtained from native Sannio (Benevento, Italy) olive cultivars grown organically and treated with Ozogea®, a corroborant also produced by Erbagil srl and based on ozonated +Oil®, an enhancer of the plants' natural defences, for a circular process in which nature cures nature. This generates a product, OZOILE®, that includes the relevant properties of the unsaponifiable portion of extra virgin olive oil, in particular the polyphenolic fraction, not involved in the ozonation process, in addition to the beneficial effects of ozone, already widely recognised by the scientific world[1,2]. The first advantage is the availability of a fairly stable form of ozone that lends itself to a wide range of medicinal applications, not only in hospitals but especially "at home". Although OZOILE® and OZOILE®-based products already have extensive scientific documentation[3,4,5] attesting to their various curative properties, other formulations with specific carriers are being studied to broaden their possible applications in the clinical field.
Natural and biodegradable polymers with pH-sensitive properties, such as alginate and chitosan, are widely used in drug delivery applications, especially in the form of biocompatible microspheres, and thermo-reactive poly(N-isopropyl- acrylamide) (PNIPAM) is frequently used in biomedical delivery systems. In this work, hydrogel multi-responsive microspheres (MRM) based on alginate, chitosan and PNIPAM were fabricated by extrusion dripping and used as an OZOILE® carrier system. Chemical composition by Fourier transform infrared (FTIR) spectroscopy, thermo-reactive properties by differential scanning calorimetry (DSC), swelling and degradation behaviour were analysed. Tests performed on MRM/OZOILE® unveiled the influence of the chitosan coating in delaying the release of OZOILE®. Thus, the results in terms of thermo-pH-responsive behaviour, swelling and degradation reveal the potential use of MRM for the therapeutic release of OZOILE®at specific inflammatory sites[6].

REFERENCES:

1. -Kaźmierczak, M.; Śmigielski, K.; Sikora, M.; Nowak, A.; Plucińska, A.; Kunicka-Styczyńska, A.; Czarnecka-Chrebelska, K.H. Molecules **2021**, 26, 3074.
2. Borges, G. Á.; Elias, S. T.; da Silva, S. M. M.; Magalhaes, P. O.; Macedo, S. B.; Ribeiro, A. P. D.; Guerra, E. N. S. J Craniomaxillofac Surg **2017**, 45, 364-370.
3. Zagolin, G.; Loss, G.; Tasinato, M.; Cassino, R.; Degli Angeli, G.; Tasinato, R. Biomed J Sci & Tech Res **2022,** 47, 38603- 38608.
4. Ciarleglio, G.; Vella, S.; Toto, E.; Santonicola, M.G. *Ceram Int* **2022** DOI: 10.1016/j.ceramint.2022.12.095.

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New G-quadruplex DNA catalysts in enantioselective sulfoxidation reaction

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DNA-based asymmetric catalysis has recently attracted increasing attention becoming a particularly interesting tool for organic chemical synthesis. G-quadruplex DNA is one of the alternative conformations that guanine-rich DNA strands can adopt and met with growing success in catalysis, being these structures able to induce sensible levels of enantioselectivity in several asymmetric reactions1. G-quadruplexes show two peculiar structural features: a central core, formed by stacked G-tetrads, that plays a very important role in recognizing planar aromatic ligands through stacking interactions, and some loops that provide the special environment of the organic reaction, affecting both the reaction rate and the enantiospecificity. The natural telomeric G-quadruplex HT21 has been extensively utilized as G4 DNA-based catalytic system for enantioselective reactions2. In order to explore the role of the residues in the loops and to improve the performances of G4 DNA catalysts, a series of HT21 analogues have been prepared, in which each sequence contains a chemically modified monomer replacing, one at a time, natural adenosines in the TTA loops with *8-bromo-2'-deoxyadenosine* (ABr), *8-oxo-2'-deoxyadenosine* (Aoxo) or *β-L-2'-deoxyadenosine* (AL) at different single loop positions. The activity and the enantioselectivity of G4 DNA metalloenzyme in the *sulfoxidation reaction* have been tested to obtain enantiomerically pure sulfoxides that have interesting potentials both in pharmaceutics3 and in asymmetric synthesis4. The substitution of an adenosine in the loops of HT21 with these modified residues has a negligible impact on the G4 DNA structural features, thermal stability and catalytic activity. Indeed, CD data strongly suggest that all modified HT21 derivatives adopt a *hybrid-type* G4 structure strictly similar to that of the natural telomeric sequence and they can catalyze a full conversion of the thioanisole substrate. On the other hand, enantioselectivity data clearly reveal the priority role of loops in inducing product chirality, since minor chemical modifications are enough to influence significantly the enantiomeric excesses obtained, considering that in most cases the DNA modified catalysts have induced lower enantioselectivities compared to the natural one (56% ee). On the contrary, the use of L-DNA is a promising strategy in modulating the enantioselectivity of a reaction, since the introduction of a single residue with opposite chirality to the rest of the sequence in specific loops consents to obtain ee values strictly comparable or also higher than those shown by the natural sequence. Particularly, the introduction of an AL residue in the first loop has significantly proved to be capable of producing about 84% enantiomeric excess, the highest enantioselectivity for DNA based oxidation reaction to date.

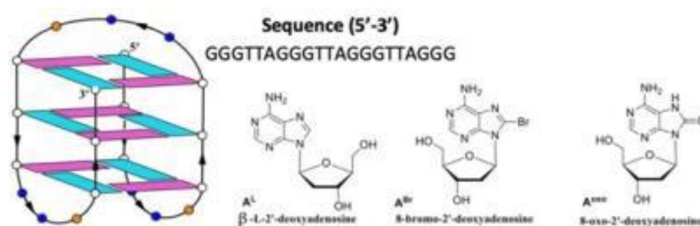

Figure 1. Sequence of HT21, schematic representation of G-quadruplex structure and chemical structures of the adenosine derivatives introduced in different loop positions.

REFERENCES:

1. Roe, S.; Ritson, D.J.; Garner, T.; Searle, M.; Moses, J.E. *Chem. Commun*. **2010**, *46*, 4309–4311. 2.
2. Li, Y.; Cheng, M.; Hao, J.; Wang, C.; Jia, G.; Li, C. *Chem. Sci*. **2015**, *6*, 5578–5585.

3. Borlinghaus, J.; Albrecht, F.; Gruhlke, M.C.H.; Nwachukwu, I.D.; Slusarenko, A.J. *Molecules*. **2014**, *19*, 12591–12618.
4. Otocka, S.; Kwiatkowska, M.; Madalińska, L.; Kiełbasiński, P. *Chem. Rev*. **2017**, *117*, 4147–4181.

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Biocatalytic Synthesis of an ibuprofen prodrug: xylitol as hydrophilization moiety

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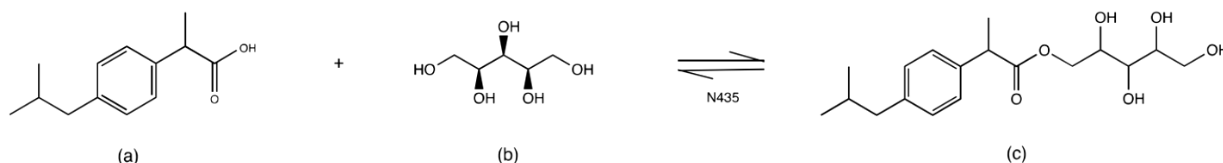
Biocatalyzed synthesis can be exploited to produce high-value products, such as prodrugs [1]. The replacement of chemical approaches with biocatalytic processes is advantageous in terms of environmental prevention, embracing the principles of green chemistry [2]. In this work, we propose the covalent attachment of xylitol to ibuprofen to produce an IBU–xylitol ester prodrug [3]. Xylitol was chosen as a hydrophilizer for the final prodrug, enhancing the water solubility of ibuprofen. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) extensively used as an analgesic, anti-inflammatory, and antipyretic [4]. Despite being the third-mostprescribed medicine in the world, the aqueous solubility of ibuprofen is just 21 mg/L [5]. This poor water solubility greatly limits the bioavailability of ibuprofen. We aimed to functionalize ibuprofen with xylitol using the reusable immobilized N435 biocatalyst. Instead of a biphasic media, we proposed a monophasic reaction environment. The characterization of the IBU–xylitol ester was performed by 1H, 13C-NMR, DEPT, COSY, HMQC, HMBC, FTIR, and MS spectroscopy. Preliminary in vitro tests showed that this enzymatically synthesized prodrug of ibuprofen reduced the expression of the interleukin 8 genes in human bronchial epithelial cells (IB3-1) from cystic fibrosis (CF) patients.



Scheme 1: Esterification scheme of (**a**) ibuprofen racemate with (**b**) xylitol, catalysed by N435 (*Candida antarctica* lipase type B, immobilized) in monophasic media; (**c**) ibuprofen xylitol ester.

REFERENCES:

1. Redasani, V. K. & Bari, S. B. *Prodrug Design: Perspectives, Approaches and Applications in Medicinal Chemistry*. *Prodrug Design: Perspectives, Approaches and Applications in Medicinal Chemistry* (Academic Press: Cambridge, MA, USA, 2015). doi:10.1016/C2014-0-05152-0.
2. Kharissova, O. V., Kharisov, B. I., González, C. M. O., Méndez, Y. P. & López, I. *Greener synthesis of chemical compounds and materials*. *Royal Society Open Science* vol. 6 (2019).
3. Zappaterra, F. *et al.* Xylitol as a Hydrophilization Moiety for a Biocatalytically Synthesized Ibuprofen Prodrug. *Int. J. Mol. Sci.* **23**, 2026 (2022).
4. Adams, S. S., Bresloff, P. & Mason, C. G. Pharmacological differences between the optical isomers of ibuprofen: evidence for metabolic inversion of the (—)-isomer. *J. Pharm. Pharmacol.* **28**, 256–257 (1976).
5. Yalkowsky, S. & Dannenfelser, R. *The aquasol database of Aqueous Solubility*. (CRC Press, 1992).

Enzymatic synthesis of ascorbic acid-ketone bodies hybrids

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The emerging strategy in the treatment of multifactorial diseases, relies on the use of multi-target drugs which integrate multiple pharmacophores through a stable or metabolizable linker, in order to administer a single molecule, able to simultaneously act at multiple sites of relevance of the pathological framework. Nowadays, it is widely known that ketone bodies as well as ascorbic acid play a central role for the maintenance of human health by the control of oxidative stress and inflammation.[1] In this respect, these compounds hold a significant promise as pro-survival substrates, for the therapy or prevention of various oxidative stress-related chronic neurological disorders and cardiovascular injuries.[2] In spite of this, ascorbic acid ketone bodies hybrids have never been proposed as alternative multicomponent and their preparation is uncovered by the literature. In virtue of its accessibility and reactivity, the hydroxyl group in position 6 of ascorbic acid have represented a privileged site for the anchoring of additional pharmacophores or other moieties.[3] Herein, we describe an enzymatic procedure that allowed the selective and complete conversion of ascorbic acid into the corresponding acetoacetyl ester (**2a**). On the other hand, the same approach applied to the synthesis of the (*R*)-3-hydroxybutyryl analogue (**3a**) led to selectivity issues due to the competition between the hydroxyl group of the short-chain ester **3** and that in position 6 of the ascorbic acid which resulted in the formation of oligomeric byproducts. This drawback, has been overcame through two strategies: **a)** the optimization of the reagents molar ratio which afforded the desired product **3a** in 57% isolated yield; **b)** by following a protection-deprotection strategy, based on the use of the methoxymethyl-protected **3** which, although an increased number of synthetic steps, allowed to completely convert the starting ascorbic acid furnishing the product **3a** with an overall yield of 90%.

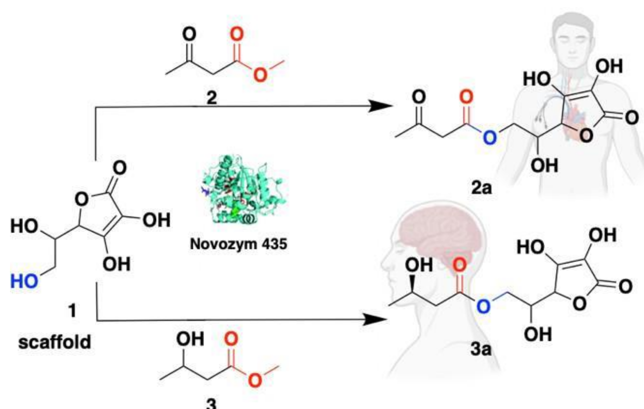

Figure 1. Schematic representation of the enzymatic synthesis and product's sites target.

REFERENCES:

1. Macan, A. M.; Gazivoda Kraljević, T.; Raić-Malić, S. Therapeutic perspective of vitamin C and its derivatives. *Antioxidants*, **2019**, *8*, 247-282.
2. Puchalska, P.; Crawford, P. A. Multi-Dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metabolism* **2017**, *25* (2), 26 –284.
3. Uesato, S.; Kitagawa, Y.; Kaijima, T.; Tokuda, H.; Okuda, M.; Mou, X. Y.; Mukainaka, T.; Nishino, H. Inhibitory effects of 6-*O*-acylated L-ascorbic acids possessing a straight- or branched-acyl chain on Epstein-Barr virus activation. *Cancer Lett*., **2001**, *166*, 143?146.

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Gruppo Interdivisionale Biotecnologie

WORKSHOP: *I chimici per le biotecnologie*

Napoli, 27 Febbraio 2023

Ferritin chemical modification for the multifunctional nucleic acid delivery

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Small interfering RNA (siRNA) represents a revolutionary tool for gene therapy with a wide array of potential applications in the regulation of gene expression.[1] However, a successful clinical application of nucleic acid-based therapy requires novel delivery options because of the extremely labile nature of siRNA under physiological conditions, which hamper its efficient and sustained delivery. To physically entrap siRNA duplexes in the inner cavity of an engineered Humanized ferritin from Archaeoglobus fulgidus (HumAfFt), piperazine-based compounds featuring one or two piperidine rings (PAs) were rationally designed and synthesized (Figure 1).[2] These rigid-rod-like amines were further functionalized with thiol-reactive crosslinkers (i.e., maleimide and fluorobenzene sulfonamide) for chemoselective conjugation of cysteine residues inside the HumAfFt cavity (Figure 1).[2] These systems allowed siRNA delivery into HeLa, HepG2 and MCF-7 cancer cells with an improved silencing effect on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression with respect to traditional transfection methodologies.[2] They provided a promising TfR1-targeting system for multifunctional siRNA delivery to therapeutic applications. Significantly, the developed siRNA-based silencing systems might be employed for various biotechnological applications.

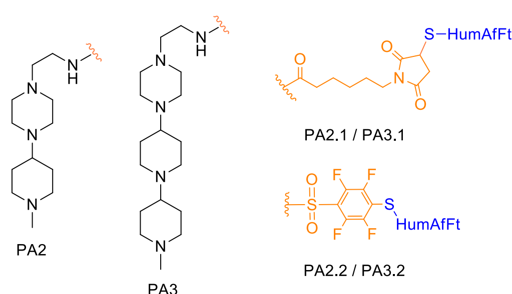

Figure 1: Piperazine-based compounds featuring one (PA2) or two piperidine rings (PA3) attached through thiol-reactive groups (PA2.1/PA3.1 or PA2.2/PA3.2) to topologically selected protein cysteine residues.

REFERENCES:

1. K. Paunovska, D. Loughrey, J. E. Dahlman Nature Reviews Genetics 2022, 32, 265 – 280.
2. N. Natalia, F. Ghirga, C. Del Plato, G. Peruzzi, C. M. Athanassopoulos, M. Mori, M. E. Crestoni, D. Corinti, F. Ugozzoli, C. Massera, A. Arcovito, B. Botta, A. Boffi, D. Quaglio, P. Baiocco Bioconjugate Chemistry 2021, 32, 1105 – 1116.

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Gruppo Interdivisionale Biotecnologie

WORKSHOP: *I chimici per le biotecnologie*

Napoli, 27 Febbraio 2023

Intrinsically emissive peptide nucleic acids

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Peptide nucleic acids (PNA) are well-knows from decades yet have been mostly used on basic academic research targeting nucleic acids substrates due to structural affinities.[1,2] PNA, DNA, and RNA share the same nucleobase alphabet allowing interactions among them. Today, PNA are clearly recognized interesting candidates for both antisense and antigene therapies, gene editing but also nucleic acid sensing and imaging.[3,4] In this scenario, the possibility to replace natural occurring nucleobases with emissive isomorphic analogs along the PNA sequences represents a cutting-edge research field which aims to overcomes limitations of the traditional nucleic acid tag with fluorophores.[5] These emissive nucleobases have shown unique emissive responsiveness to variation in the environmental pH and polarity and biological relevant events like duplex formation.[6]

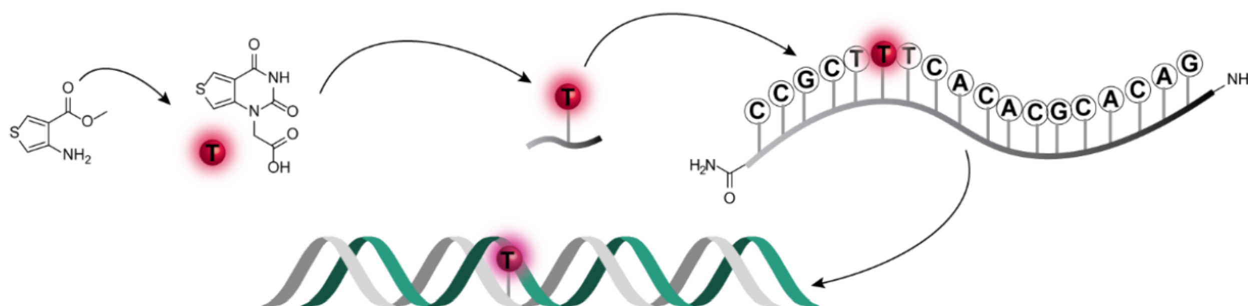

Figure 1. Intrinsically emissive PNA.

Herein we present the synthesis, characterization and successful further incorporation into model PNA standard sequences of a thieno[3,4-d]pyrimidine fluorescent analog of thymine to provide intrinsically emissive PNAs. The photophysical features of the emissive PNAs, as well as the ability to form stable heteroduplex with complementary DNAs have been evaluated by absorption and emission spectroscopies and micro-differential scanning calorimetry. The preliminary results suggest that the replacement of one natural thymine with an emissive analog had a minimal impact on the DNA-PNA heteroduplex stability while conferring to the PNA remarkable brightness and modular luminescence that can be a useful feature in chemical biology assays.

REFERENCES:

1. Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624-630.
2. Saarbach, J.; Sabale, P. M.; Winssinger, N. *Curr. Opin. Chem. Biol.* **2019**, *52*, 112-124.
3. Suparpprom, C.; Vilaivan, T. *RSC Chem. Bio.* **2022**, *3*, 648-697.
4. Brazil, R. *ACS Cent. Sci.* **2023**, *9*, 3-6.
5. Sinkeldam, R. W.; Greco, N.; Tor, Y. *Chem. Rev.* **2010**, 110, 2579-2619.
6. Rovira, A. R.; Fin, A.; Tor Y. *Chem. Science*, **2017**, *8*, 2983-2993.

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Flow biocatalyzed reactions for the preparation of valuable aglycones from the corresponding natural glycosides

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Agri-food waste is a significant environmental and economic problem as tons of residues (seeds, peels, leaves, branches, trunks, and roots) which are typically unexploited, need to be managed and disposed.

Green technologies, such as biocatalysis, can be used to obtain natural valuable compounds with attractive biological properties starting from the above-mentioned wastes. In this context, hesperidin and rutin, glycosides typically found in citrus species and their residues, [1] seem to be noteworthy since their aglycones, hesperetin and quercetin respectively, present interesting biological activities as antioxidants, anti-inflammatory and antiarrhythmic agents. [2,3]

Through a cascade reaction employing a commercial α-rhamnosidase and an extremophilic glycosidase 4 from *Halothermothrix orenii* (HOR) produced in our lab, the aglycones were obtained (Fig.1). The biotransformations were firstly optimized in batch mode selecting a biphasic medium (water/TMO) as the best option to both enhance the substrate solubility and to obtain highly-productive processes.

To maximize the overall yields, while increasing the process sustainability, in continuous processes have been developed through enzyme immobilization techniques and flow chemistry reactors. 5,6 After the optimization of the single-step reactions the enzymes have been simultaneously immobilized on the same carrier (glyoxyl-agarose) to obtain a one-pot, 2-step flow reaction. Finally, an in-line extraction was added downstream the process further enhancing the process automation.

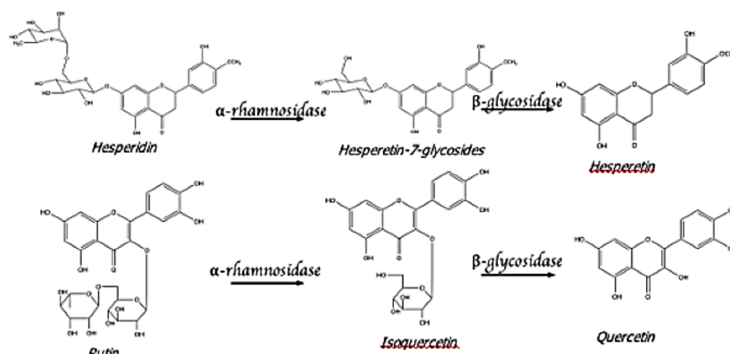

Figura 1, Enzymatic reactions from hesperedin to hesperetin and from rutin to quercetin.

REFERENCES:

1. Dakshini, K. *Journal of Chemical Ecology* **1991** 17(8).
2. Paez-Ribes, M., et al., *EMBO Molecular Medicine,* **2019** 11(12).

3. Alvarez-Collazo, J., et al., *British Journal of Pharmacology*, **2019** 176(8), 1090–1105. 0
4. Bourne, Y., et al., *Current Opinion in Structural Biology*, **2001** 11(5), 593–600.
5. Tamborini, L., et al., *Trends in Biotechnology.* **2018** 36(1), 73-88
6. Benítez-Mateos, A. I., et al., *Reaction Chemistry & Engineering.* **2021, 6(4), 599-6**

Società Chimica Italiana
Gruppo Interdivisionale
Biotecnologie

Plants from the Myrtaceae family as promising sources for biological applications: bio-guided isolation of alkylphloroglucinol glucosides

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Myrtaceae, the ninth largest family of flowering plants, are a valuable source of bioactive specialized metabolites. In particular, phloroglucinols derivates - the most extensively studied specialized metabolites from Myrtaceae- have become attractive targets for organic chemists, thanks to their structural features and exhibited a wide range of biological and pharmacological properties above all antimicrobial activity (1). Recent decades, in fact, have witnessed a remarkable increase in studies reporting antimicrobial compounds against pathological microorganisms isolated from Myrtaceae species (1): just to name a few phloroglucinols, myrtocummulone A from *Myrtus communis*, callistrilone A from *Callistemon lanceolatus* (2), eugenials C and D from *Eugenia umbrellifora* (3). Despite of many studies about phloroglucinols, there are few papers about alkylphloroglucinol glycosides.

Now, we reports the bio-guided phytochemical investigation of glycosidic fractions of *Myrcianthes cisplatensis* leaves, collected in Arizona, and *Myrtus communis* growing in Sardinia. Methanolic crude extracts, investigated by 2D-NMR in order to furnish information about the specialized metabolite content, were tested for their antimicrobial activity against two strains of *Staphylococcus aureus:* ATCC 29213 and 43300 (a methicillin-resistant *Staphylococcus aureus* strain, MRSA). Based on the promising results both extracts were subsequently fractionated using chromatographic techniques. From *M. cisplatensis* were isolated for the first time three cinnamoylated alkylphloroglucinol glucosides, named *p*-coumaroylmyrciacommulone A-D (figure 1), while gallomyrtucommulones G – H and myrtucommulonoside (figure 1), were characterized for the first time from *M. communis* (4). Their structures were elucidated through spectroscopic techniques: 2D-NMR experiments (HSQC, HMBC, HSQC-TOCSY) and spectrometric analyses (HR-MS). The antimicrobial assessment of pure compounds has been evaluated.

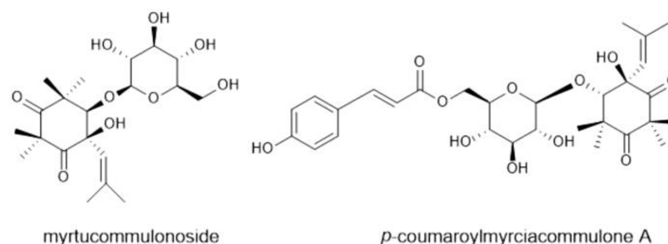

Figure 1. Alkylphloroglucinol glucosides from *M. communis* and *M. cisplatensis*.

REFERENCES:

1. Celaj, O.; Duràn, A.G.; Cennamo, P.; Scognamiglio, M.; Fiorentino, A.; Esposito, A.; D'Abrosca, B.. *Phytochem Rev.* **2020**, *20*, 259-299.
2. Cao, J.Q.; Huang, X.J.; Li, Y.T.; Wang, Y.; Wang, L.; Jiang, R.W.; Ye, W.C. *Org. Lett.* **2016**, *18*, 120-123.
3. Faqueti, L.G., Farias IV., Sabedot E.C.; Delle Monache F.; San Feliciano A.; Schuquel I.T.; Cechinel-Filho V.; Cruz A.B.; Meyre-Silva C.; *J Agric Food Chem*, **2015**, 63, 8151-8155.
4. 4. Guzzo, F.; Durán, A.G.; Sanna, C.; Marasco, R.; Molfetta, N.; Buommino, E.; Fiorentino, A.; D'Abrosca, B.. *Molecules,* **2022**. 27.

ISASI-CNR Naples Cryo Electron Microscopy laboratory

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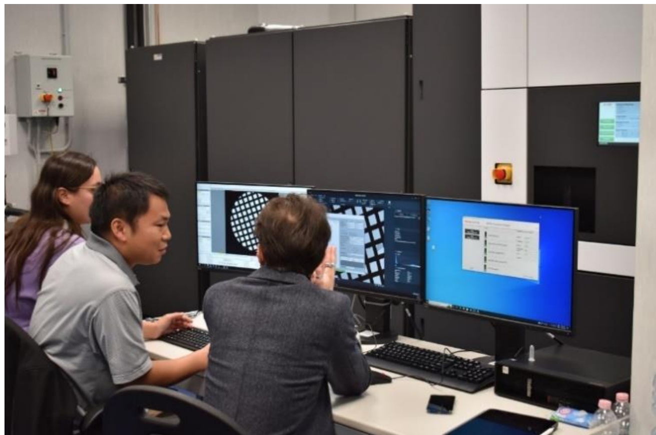
The Cryo Electron Microscopy laboratory at ISASI Naples provide the possibility to identify, visualize and characterize biological macromolecules alone and in their cellular environment. The Cryo-EM lab is designed to efficiently combine different work flows for single-particle analysis (SPA), that provides the high-resolution 3D structure of proteins, enzymes, other macromolecules, nanoparticles and electron tomography (ET), which is used to observe and measure the inner architecture of cells, tissues and organoids.
An essential part of the work flow for SPA and ET includes plunge freezing and lamella preparation to observe vitrified samples using focused ion beam – scanning electron microscopy (FIB-SEM) technology.
The Cryo-EM lab can also provide Material Science analysis and sample fabrication by (FIB-SEM) technology.
The Cryo-EM lab is equipped with the state-of-art electron microscope: Thermo Scientific Glacios 200kV TEM equipped with Thermo Scientific Falcon 4 camera and Selectris X imaging filters; Thermo Scientific Aquilos 2 Dual Beam cryo-FIB system; Thermo Scientific Vitro-bot.
We show our first results made on proteins, nanoparticles and yeast cells lamella.



Pictures of the Cryo EM lab: Glacios (left side) and Aquilos2 (right side).

Exploiting the Optical Tweezer technology to reveal thymidylate synthase interactions with its consensus mRNA

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Human Thymidylate Synthase (*h*TS) is a critical target for cancer therapy, as it is the key enzyme of folate cycle for DNA replication and repair. However, drug resistance in colorectal cancer (CRC) cells limits the clinical efficacy of TS-targeting drugs. Cells with increased TS protein activity are less sensitive to TS-targeting chemotherapeutic agents (5-FU and pemetrexed), and exhibit cross-resistance to other chemotherapeutics (e.g., oxaliplatin). For this reason, we have recently proposed innovative hTS inhibitors with an innovative mechanism of action that prevent protein overexpression and downregulate hTS by increasing its proteasomal degradation rate, by targeting the dimer interface and drive the dimer to separate into its monomers (dimer destabilizers, DDIS). We have demonstrated that after the administration of DDIS, both *in vitro* and *in vivo*, hTS catalytic activity is inhibited, and the intracellular protein level is rapidly decreased because of TS dimer dissociation[1]. *h*TS, as an RNA binding protein, is also able to control its own biosynthesis with a negative feedback mechanism on its mRNA, by exploiting a peripheric mRNA binding domain (RBD). Through RBD, hTS is also assumed to regulate cellular expression of several genes, including those regulating apoptosis and chemosensitivity (c-myc, blc-2, p53)[2]. The state of ligand occupancy influences hTS's RNA-binding activity. When hTS is devoid of active-site ligands, its RNA-binding activity is at its peak, resulting in translational repression of TSmRNA. When hTS is bound to either of its physiologic substrates or substrate-analogue inhibitors, the *in vitro* binding of TS to its mRNA is reduced[3]. The aim of this work is thus to study the translational repression mechanism of hTS on its mRNA with Optical Tweezers (OT), an innovative biophysical tool which measure the mechanical energy associated with the interaction between macromolecules. For this purpose, we have synthesized a truncated domain of TS mRNA binding site 1, whose structure folding was predicted with UNAFold (37°C, 1M NaCl). The computational results suggest the formation of a hairpin structure with three possible configurations. Then we performed the mRNA fragment (110 bases) synthesis, which was obtained by PCR from a pBR322 plasmid clone. The fragment was functionalised with digoxigenin through an extension of the handle using DIG-dUTP and annealed to a biotin-streptavidin recognition system to form the final construct (handle A-dig/handle B-biotin/ TSmRNA hetero handle). The construct will be fixed into the optical trap, and by repeated stretching and refolding, we will gain insights into the energies associated with mRNA actual folding. Consequently, by adding a stoichiometric amount of recombinant hTS, we will reveal the actual regions of mRNA interactions with the protein. This will provide to understand the translational repressor's activity of the protein and characterized which form of hTS is important to interact with its mRNA between active/inactive or monomeric/dimeric conformation. This may help us in the study of new possible mechanism of action of new inhibitors by unraveling non exposed pockets and grooves which are physiologically hidden, this tool is suitable to develop alternative medicinal chemistry strategies.

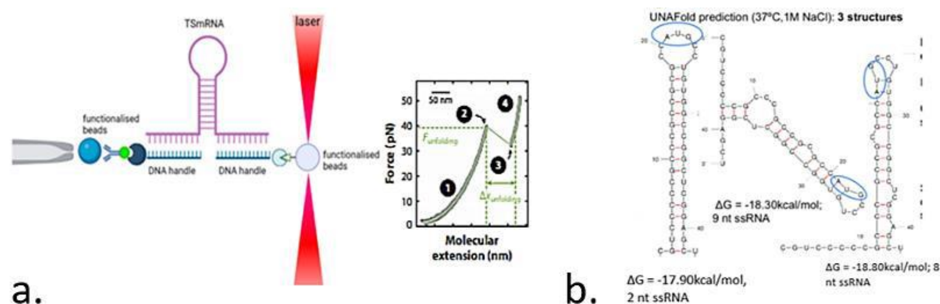

Figure 1. (a) Schematic mechanism of an Optical Tweezer experiment. (b) UNAFold prediction of TS mRNA folding.

REFERENCES:

1. Luca Costantino et al, **2022**. *Destabilizers of the thymidylate synthase homodimer accelerate its proteasomal degradation and inhibit cancer growth. eLife* **11**:e73862.
2. Salo-Ahen, O. M. H. & Wade, R. C. The active-inactive transition of human thymidylate synthase: Targeted molecular dynamics simulations. *Proteins Struct. Funct. Bioinforma.* **79**, 2886–2899 (2011).
3. Ackland, S. P., Beale, P. & Peters, G. J. Thymidylate synthase inhibitors. *Cancer Chemother. Biol. Response Modif.* **21**, 1–28 (2003)

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Napoli, 27 Febbraio 2023

Modification of halloysite lumen with dopamine derivatives as filler for antibiofilm coating

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Development of nanocomposite coating with antibiofilm properties is of fundamental importance to efficient fight biofilm formation preventing infections in biomedical area. In this context, halloysite nanotubes (HNTs), biocompatible and low-cost clay mineral, have been efficiently used as filler for different polymeric matrices affording several nanocomposites with appealing antimicrobial properties.[1] The modification of HNTs surfaces represents a valuable strategy to improve the utilization of the clay for biological purposes.[2] Herein, the covalent modification of the HNTs lumen with properly designed dopamine derivatives with different perfluoroalkyl chain length is reported. The obtained nanomaterials are thoroughly characterized by several techniques which prove the selectivity of the modification and the increased hydrophobicity of the lumen. In particular ^{27}Al solid state NMR spectra showed a downfield shift of the Al signal. As proof of concept the antibiofilm properties on *E. coli* strain of the nanomaterials are assayed as well obtaining different results according to the length of perfluoroalkyl chains of organic molecules as found by ^{19}F solid state NMR spectra. Finally, the introduction of the HNTs fillers into a polydopamine matrix allows for the preparation of functional coatings, resistant to formation of microbial biofilms, that could find applications for example on medical implants.

REFERENCES:

1. Massaro, M.; Ciani, R.; Cinà, G.; Colletti, C. G.; Leone, F.; Riela, S. *Antibiotics* **2022**, *11*, 1761.
2. Alfieri, M.L.; Massaro, M. d'Ischia, M.; D'Errico, G.; Gallucci, N.; Gruttadauria, M.; Licciardi, M.; Liotta, L.F.; Nicotra, G.; Sfuncia, G.; Riela, S. *J. Coll. Interf. Sci.* **2022**, *606*, 1779-1791.

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Proteolytic enzymes as a powerful tool for the recovery and biocatalytic conversion of agri-food biomasses

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The increase in the world's population, which is projected to grow to 8.5 billion in 2030, is reflected in a sharp increase in the demand for food and feed. At the same time the use of sustainable land management practices is encouraged. All these aspects are reflected in the UN 2030 Agenda for Sustainable Development, in Goal 2 (zero hunger). According to the data provided, a third of the world's food is wasted, yet 821 million people are undernourished. Therefore, a lot of research is aiming to the development of biorefinery processes capable of recovering food waste, by-products, and co-products of the food processing industry through the sequential extraction and valorisation of the different fractions (lipids, proteins, fibres, antioxidants, etc.), with the goal of eliminating waste and increasing food production in a sustainable way. The protein fraction of food-derived biomasses can be recovered with a wide variety of extraction methods, optimized according to the type of raw material, the intended applications, and the industrial feasibility of the process. Obviously, the trend is to move towards new technologies with a lower environmental impact than conventional techniques. In this perspective, enzyme-assisted extraction is a very promising technique for extracting proteins from a wide variety of raw materials, both from vegetable and animal processing waste [1, 2, 3]. The use of proteolytic enzymes during the extraction has many advantages, such as higher protein solubilization, better protein digestibility and higher extraction yield. Many of these benefits are due to the catalytic bioconversion of proteins into shorter peptides. The small size of the peptides in fact makes the final product completely soluble in water, and the proteolysis that takes place during extraction increases the degree of hydrolysis, favouring digestion. Furthermore, enzymatic hydrolysis is also a promising tool for reducing the allergenicity of proteins, a very important aspect for both traditional and new protein sources [4]. The versatility of enzymatic bioconversion allows the setup of biorefinery processes by fine-tuning parameters such as enzyme type, enzyme/substrate ratio, hydromodule, temperature, and extraction time.

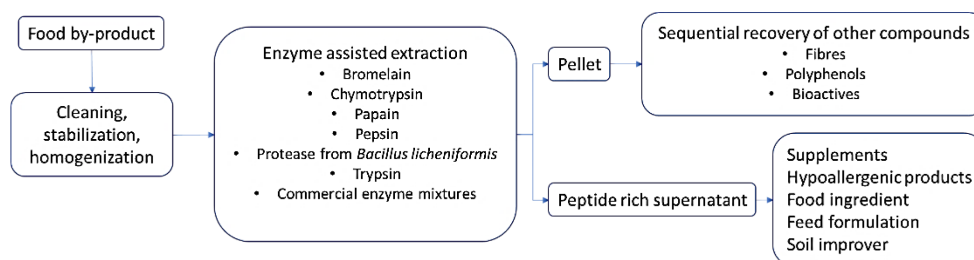

Figure 1: enzyme assisted protein extraction in a biorefinery approach.

REFERENCES:

1. Tedeschi et al. (2022). A novel approach based on enzymatic hydrolysis for the valorisation of edible Parmigiano Reggiano cheese rinds. International Dairy Journal, 134, 105454.
2. Prandi et al. (2021). Extraction and chemical characterization of functional phenols and proteins from coffee (Coffea arabica) by-products. Biomolecules, 11(11), 1571.
3. Prandi et al. (2021). Targeting the nutritional value of proteins from legumes by-products through mild extraction technologies. Frontiers in Nutrition, 8, 695793.
4. Calcinai et al. (2022). Effectiveness of enzymatic hydrolysis for reducing the allergenic potential of legume by-products. Scientific Reports, 12(1), 16902.

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WORKSHOP: *I chimici per le biotecnologie*

Napoli, 27 Febbraio 2023

Mass spectrometry study of Thioredoxin System targeted by N-Heterocyclic Carbene (NHC)Gold(I) complexes

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The Trx system is involved in the cellular redox regulation network. Thioredoxin (Trx) and Thioredoxin Reductase (TrxR) enzymes are essential constituents of this apparatus. Elevated expression of thioredoxin is associated with increased proliferation of tumour cells and inhibition of apoptosis; therefore specific inhibitors are developed to target TrxR/Trx for cancer treatment [1]. Redox active sites of Trx and TrxR are respectively constituted, by two cysteine (WCGPCK) at the N-*terminus* and by a selenol thiol motif (GCUG, where U=SeCys) at the C-*terminus*.

Soft Lewis acid Au(I) based compounds have high affinity for Lewis soft base Cys and SeCys therefore both side chain of aminoacids can be coordinated inhibiting the redox activity. In this context, while TrxR has been largely explored as target of cytotoxic gold complexes [2], Trx has been neglected. Here, we present the investigation by mass spectrometry of interaction with Trx of two $(NHC)_2$-gold(I) complexes (Figure 1), able to cross mitochondrial membrane, in comparison with the Au(I) drug auranofin.

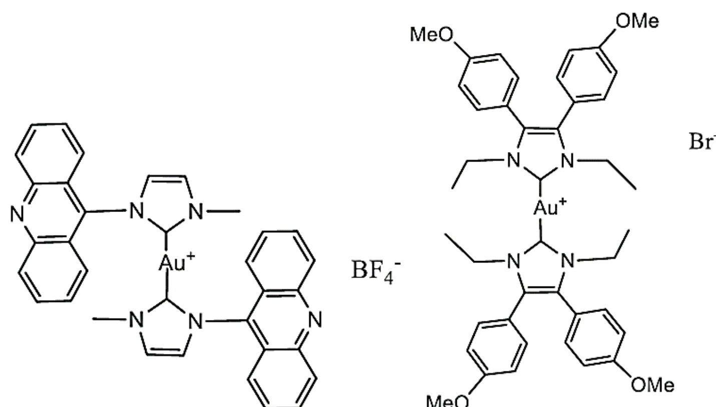

Figure 1*: Chemical structures of two $(NHC)_2$-gold(I)complexes*

Acknowledgements*: Authors thank CNR for funding the Bioinorganic Drugs joint laboratory: A multidisciplinary platform promoting new molecular targets for drug discovery.*

REFERENCES:

1. Zhang, J.; Li, X.; Han, X.; Liu, R.; Fang, J. *Trends Pharmacol. Sci.* **2017**, *38*,794-808.
2. Bindoli, A.; Rigobello, M.P.; Scutari, G.; Gabbiani, C.; Casini, A.; Messori, L. *Coord. Chem. Rev.***2009**, *253*, 1692-1707.

LC-MS/MS proteomics for the rapid and selective screening of drug resistances in *Leishmania infantum* clinical isolates

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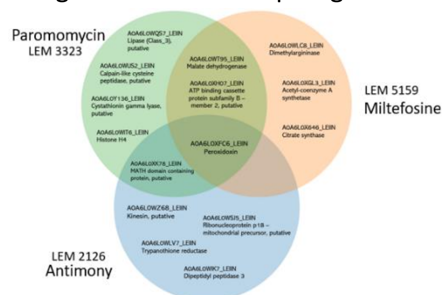
Vector borne diseases (VDBs) are the cause of more than 75% of the emerging human infections worldwide originated from animals. Severe changes in the atmospheric climate conditions, along with the upcoming of globalization and world-wide trade exchanges, have contributed to the spread of this infection outside the African Continent to the south European areas. Mass Spectrometry (MS) studies to rapidly detect bacterial infections and drug resistance in the human/animal bodies and environment, can also be proposed for the detection of rapidly incoming and less known parasitic diseases. One of the most diffused VDB is represented by Leishmaniasis, represented by over 12m of new clinical cases every year worldwide afflicting both humans and dogs, one third of which are hit by recrudescence during their therapeutic treatment. Depending on the endemic region of diffusion and the specific Leishmania strain considered, current therapeutic options include miltefosine, antimonials and paromomycin. The unsupervised usage of those or similar drugs in the livestock and humans has selected specific hyper-resistant strains, determining the rapid onset of severe drug resistant issues. This leads to decrease of drugs efficacy and increase of the risk of diffusion of the infections.[1] For this reason, in the present work we investigate through MS the potential application of an highly sensitive assay based on the study of proteome modulation of different Leishmania infantum strains characterized by drug resistance using as a model, THP-1 cells *in vitro*. We have treated the THP-1 cell lysates with a well-established FASP protocol to extract and hydrolyse proteins, which were analysed with LC-MS/MS bottom-up proteomics[2]. The quali-quantitative differential analysis of the samples performed with Mascot and Progenesis (Waters) against sample controls (non-resistant lines) revealed the presence of 15 differentially Expressed Proteins (DEP's), 6 of which in miltefosine sample, 8 in paromomycin and 6 in Sb(V) resistant strain. Some DEPs are mutual to more than one lines, and peroxidoxin - whose role in parasitic oxidative stress neutralization is well established - resulted up-regulated (FC >2) in all the three resistant lines. The modulation of these proteins could exploit the rapid determination of drug resistances patterns from clinical patients, to correctly evaluate the most promising therapeutic regimen and avoid the genetic selection of further resistances. Also, these biomarkers could be extended to dogs infected with Leishmaniasis for an early detection of the infection. Another important outcome of the present work is the identification of perspective targets for new therapy against drug resistant parasites.

We thank the COST Action OneHealthdrugs CA21111 for inspiring the research development.



REFERENCES:

1. Kaye, P et al. Leishmaniasis: complexity at the host–pathogen interface. *Nat Rev Microbiol* **9**, 604–615 (2011).
2. Tagliazucchi, L et al. Label free Mass spectrometry proteomics reveals different pathways modulated in THP-1 cells infected with therapeutic failure and drug resistance Leishmania infantum clinical isolates. Accepted on *ACS Inf Dis*, Jan **2023.**

Organs-on-chip as new frontiers for disease modelling and personalized medicine

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The introduction of bidimensional (2D) cell cultures in preclinical research represented a milestone in the development of novel studies on the physiological and pathological processes that characterize living beings. These systems, although easy to handle and economically advantageous, presented some big limitations, mainly due to the lack of a three-dimensional complex structure, with the consequent loss of most of the biological information that derive from the spatial architecture of complex systems. For this reason, in the last decades, many techniques for the in vitro maintenance of three-dimensional cell structures have been developed, namely 3D cell cultures, which allowed for significant improvements in the analysis of tissutal morphological properties and cell proliferation, response to external stimuli, differentiation, drug metabolism and sensitivity, protein synthesis. Moreover, the introduction of in vitro microperfusion systems, capable of mechanically replicating the bloodstream on artificial supports, led to the creation of organ-on-a-chip (or organ chips) platforms, which represented an advancement, in terms of reproducibility of tissue physiological milieu, respect to spheroids or organoids cultured on organic gels. Organ-on-a-chip systems also allow real time, high-definition graphical analysis of biochemical, genetic e metabolic activities of human tissues, both in healthy and pathological conditions; all this is achieved by preserving, during the whole experimental procedure, the complex biological interactions network between the different components of human organs. Up to date, many tissues have been successfully modelled on organ-on-a-chip platforms: kidney tubules, small intestine, bronchioles, liver, brain blood barrier, pulmonary alveoli, and bone marrow. In addition, organ-on-a-chip models can also be adopted to accurately study organ responses to different stimuli like drugs, radiations, cigarette smoke and pathogens. Lastly, by modelling diseased tissues on organ-on-a-chip devices, researchers were able to explore novel therapeutic strategies and drug development for multiple pathological conditions, among which thrombosis, inflammatory bowel disease, asthma and Barth syndrome.

Given the wide horizons of application opened by these novel biotechnologies, preclinical research is lately focusing on the constant setting-up of ad-hoc supports, capable of selectively improving the modeling strategies for healthy tissues and pathologies. Pursuing this research direction, in IRCCS Synlab SDN we are setting up, through interdisciplinary approaches, pathology models strictly resembling *in vivo* conditions, by exploiting organic matrices or engineered cell culture platforms, both currently on the market or customized. With a focus on studying cardiovascular and oncological diseases, the application of these biotechnologies is aimed at the development of artificial supports for the study of the pathophysiological mechanisms of multiple human diseases with a high social impact, as well as at the analysis of compounds of diagnostic interest, with the goal of defining new strategies for personalized medicine.

Handcrafted Metal Enzymes Poised for Biotechnology Revolution

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Thanks to their metal core, metalloproteins play a central role in some of the most difficult transformations in nature. For this reason, they are an invaluable tool in many industrial biotech applications, from denim aging to agricultural feed valorisation[1]. Over the last years, also supported by the recent advances in computational protein design, we have designed several custom-made enzymes, setting several milestones in the field[2]. By different approaches, we designed small, yet functional, models bearing multiple metal sites. In this contribution, some of them will be showcased, highlighting their potential in industrial biotechnology. We first show that by asymmetrization of the heme environment and precise positioning of the residues at the distal site, with Fe-Mimochrome 6*a (FeMC6*a), we reached unprecedented selectivity in hydrogen peroxide activation, leading to a peroxide sensor and to the smallest decolorization enzyme[3]. Then, we show how very small changes, like cobalt replacement in CoMC6*a, may induce a dramatic shift in catalytic activity, towards a high-pH insensitive artificial hydrogenase[4]. Finally, we describe our efforts to couple a photosensitizing zinc porphyrin with a 3kDa iron-sulphur designed protein, leading to the first fully artificial light-harvested electron cascade[5]. In perspective, our designed metalloproteins will be equipped for the biotechnology revolution.

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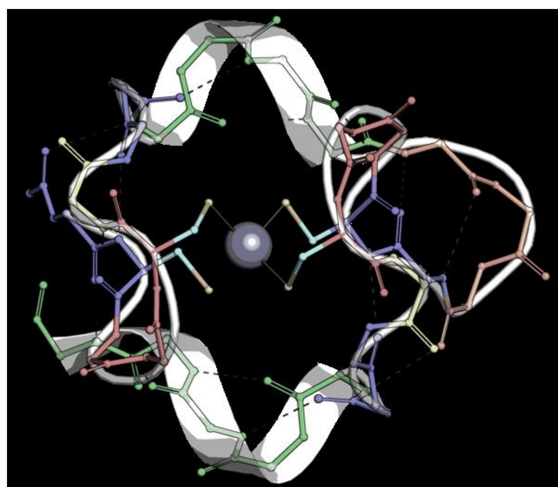

REFERENCES:

1. Arbige, M. V.; Shetty, J. K.; Chotani, G. K. Industrial Enzymology: The Next Chapter. *Trends in Biotechnology* **2019**, *37* (12), 1355–1366.
2. Leone, L.; Chino, M.; Nastri, F.; Maglio, O.; Pavone, V.; Lombardi, A. Mimochrome, a Metalloporphyrin-Based Catalytic Swiss Knife. *Biotechnology and Applied Biochemistry* **2020**, *67* (4), 495–515.
3. Zambrano, G.; Nastri, F.; Pavone, V.; Lombardi, A.; Chino, M. Use of an Artificial Miniaturized Enzyme in Hydrogen Peroxide Detection by Chemiluminescence. *Sensors* **2020**, *20* (13), 3793.

4. Le, J. M.; Alachouzos, G.; Chino, M.; Frontier, A. J.; Lombardi, A.; Bren, K. L. Tuning Mechanism through Buffer Dependence of Hydrogen Evolution Catalyzed by a Cobalt Mini-Enzyme. *Biochemistry* **2020**, *59* (12), 1289–1297.
5. Chino, M.; Costanzo, L. D.; Leone, L.; Gatta, S. L.; Famulari, A.; Chiesa, M.; Lombardi, A.; Pavone, V.
Designed Rubredoxin Miniature in a Fully Artificial Electron Chain Triggered by Visible Light., **2022**. Preprint: dx.doi.org/10.21203/rs.3.rs-1473985/v1

Società Chimica Italiana
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Cationic calix[4]arenes as tools for the intracellular delivery of microRNA mimics and antimiR peptide nucleic acids

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The modulation of intracellular microRNAs (miR) levels by means of (synthetic) oligonucleotides is a promising strategy for the treatment of cancer, genetic diseases, and viral infections.[1] Depending on their structure and sequence, these molecules can in fact restore the expression of both down- and upregulated miRs, correcting detrimental mechanism that govern the studied pathologies. However, the implementation of these approaches into clinical stages is often impaired by the difficult transport of the tested oligonucleotide through target cell membranes. This task is generally performed by conjugation with carrier molecules (e. g. peptides, saccharides, and others), or by using transfecting agents or multicomponent nanoparticles, often requiring challenging protocols or resulting in significant toxicity. Hence, the development of new classes of efficient, biocompatible vehicles is a subject of great interest for miR therapeutics.[2]
The tetraargininocalix[4]arene **Arg-Hex** (**Scheme 1a**) proved effective in this field, performing the delivery of oligonucleotides that can act as miR mimics or antimir agents.[3] We thus decided to extend this strategy to other cationic calix[4]arenes, testing their ability to deliver a premiR RNA or an antimiR peptide nucleic acid (PNA) into HT29 and U251 cell lines. As inferred by FACS and PCR analyses, three derivatives (**Scheme 1b**) successfully delivered the cargoes into target cells, with an efficiency comparable to that of reference **Arg-Hex**. Also, MTT assays after 24 and 72 h of incubation excluded significant toxic effect in the tested conditions, further demonstrating the suitability of the tested compounds as vehicles for (artificial) oligonucleotides.

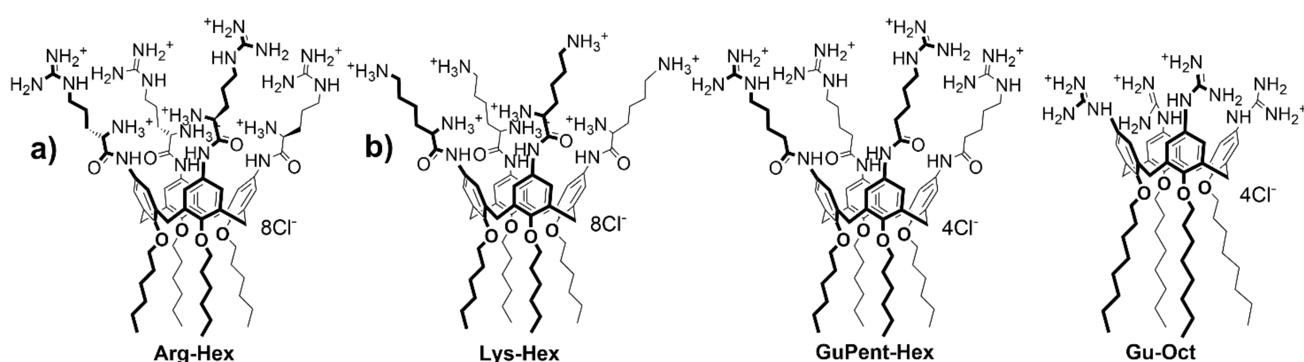

Figure 1: a) reference and **b**) novel cationic calix[4]arene tested for the delivery of a premiR RNA or an antimir PNA into cancer cell lines.

REFERENCES:

1. Fu, Z.; Wang, L.; Li, S.; Chen, F.; Au-Yeung, KK.; Shi C. *Front Pharmacol.* **2021**, *12*, 1-18.
2. Roberts, T. C.; Langer, R.; Wood, M. J. A. *Nat. Rev. Drug Discov.* **2020**, *19*, 673–694.
3. Gasparello, J.; Lomazzi, M.; Papi, C.; D'Aversa, E.; Sansone, F.; Casnati, A.; Donofrio. G.; Gambari, R.; Finotti, A. *Mol. Ther. Nucleic Acids*. **2019**, ***18***, 748–763.

Insight into the stereoselective synthesis of (1*S*)-nor(pseudo)ephedrine analogues by biocatalytic cascades

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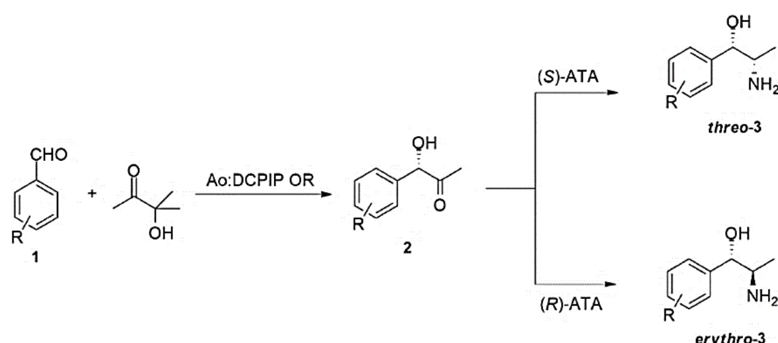
Nor(pseudo)ephedrine (N(P)E) isomers belong to the amphetamine family of ephedra alkaloids and can be found in different plants such as khat (*Catha edulis*) or Ephedra species. Besides being biologically active molecules with sympathomimetic activity, N(P)Es can be used as intermediates for the synthesis of APIs, or as auxiliaries and ligands in asymmetric organic synthesis1,2.
Due to the presence of two chiral centres, their conventional chemical asymmetric syntheses often involve long multi-step procedures, frequently with the aid of expensive and harmful metal catalysts, in which high yields and optical purities are very difficult to achieve overall. Moreover, most of these methods require several steps for intermediate isolation and purification, thus significantly increasing E-factor, solvent demands, and energy consumption3.

A two-steps biocatalytic cascade for the preparation of (1*S*)-N(P)E analogues was therefore designed and carried out, consisting of a benzoin-type condensation catalysed by the (*S*)-selective acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) and a reductive amination mediated by either a (*S*)- or (*R*)-selective amine transaminase (ATA). In order to provide reference material for assessing the performance of the biocatalyzed reactions, a multistep chemical synthesis of racemic N(P)Es was also optimized. By producing the desired products with acceptable yields and good diastereo- and enantiomeric excesses, the novel bienzymatic synthesis paved the way for a more environmentally friendly manufacturing process for such important building blocks.
Figure 1. Aim of work: two-steps biocatalytic cascade for the preparation of (1*S*)-N(P)E analogues (**3**)



REFERENCES:

1. Flavahan, N.A. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 432–439
2. Sehl, T.; Maugeri, Z.; Rother, D. *J. of Mol. Catal. B: Enzym.* **2015**,*114*, 65–71.
3. Lee, H.K.; Kang, S.; Choi, E.B. *J. Org. Chem.* **2012**, *77*, 5454–5460

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Napoli, 27 Febbraio 2023

Bioluminescence Sensing in 3D Spherical Microtissues for Multiple Bioactivity Analysis of Environmental Samples

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The development of predictive *in vitro* sensing tools able to provide rapid information on the different bioactivities of a sample is of pivotal importance, not only to monitor environmental toxicants, but also to understand their mechanisms of action on diverse molecular pathways. This mechanistic understanding is highly important for the characterization of toxicological hazards, and for the risk assessment of chemicals and environmental samples such as surface waters and effluents. Prompted by this need, we developed and optimized a straightforward bioluminescent multiplexed assay which enables the measurement of four bioactivities, selected for their relevance from a toxicological perspective, in bioluminescent microtissues. The assay was developed to monitor inflammatory, antioxidant, and toxic activity, and the presence of heavy metals, and was successfully applied to the analysis of river water samples, showing potential applicability for environmental analyses. The assay, which does not require advanced equipment, can be easily implemented in general laboratories equipped with basic cell culture facilities and a luminometer.[1]

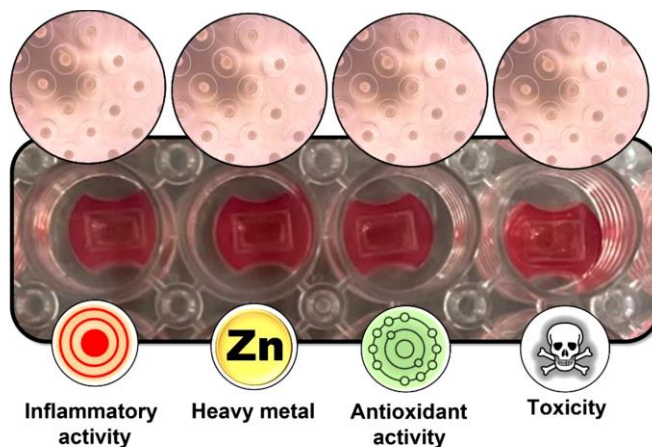

Figure 1. *Schematic representation of the bioluminescence sensing platform relying on 3D spherical microtissues for multiple bioactivity analysis*

REFERENCES:

1. Calabretta, M.M.; Gregucci, D.; Guarnieri T.; Bonini M.; Neri, E.; Zangheri, M.; Michelini, E. *Sensors.* **2022**, *22*(12), 4568.

Hydroxamate-based peptides as iron(III) chelators for antimicrobial applications

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Pathogenic bacteria synthesize iron-chelating entities known as siderophores to sequestrate ferric iron from host organisms in order to colonize and replicate.[1] The development of antimicrobial peptides (AMPs) conjugated to iron chelators represents a promising strategy for reducing iron availability, inducing bacterial death, and enhancing simultaneously the efficacy of AMPs.[2] Herein, we designed, synthesized, and characterized three hydroxamate-based peptides **Pep-cyc1**, **Pep-cyc2**, and **Pep-cyc3**, derived from a cyclic temporin L peptide (**Pep-cyc**) developed previously by some of us.[3] The Fe3+ complex formation of each ligand was characterized by UV-visible spectroscopy, mass spectrometry, IR, and NMR spectroscopies. In addition, the effect of Fe3+ on the stabilization of �-helix conformation of hydroxamate-based peptides and the cotton effect were examined by CD spectroscopy.[4] Moreover, the antimicrobial results obtained *in vitro* on some Gram-negative strains (*K. Pneumoniae* and *E. coli*) showed the ability of each peptide to chelate efficaciously Fe3+ obtaining a reduction of MIC values in comparison to their parent peptide Pep-cyc. Our results demonstrated that siderophore conjugation could increase the efficacy and selectivity of AMPs used for the treatment of infectious diseases caused by Gram-negative pathogens.

REFERENCES:

1. Kramer, J.; Özkaya, Ö.; Kümmerli, R. *Nat. Rev. Microbiol.* **2020**, *18*, 152-163.
2. Boyce, J. H.; Dang, B.; Ary, B.; Edmondson, Q.; Craik, C. S.; DeGrado, W. F.; Seiple, I. B. J. *Am. Chem. Soc.* **2020**, 142, 21310-21321.
3. Bellavita, R.; Casciaro, B.; Di Maro, S.; Brancaccio, D.; Carotenuto, A.; Falanga, A.; Cappiello, F.; Buommino, E.; Galdiero, S.; Novellino, E.; Grossmann, T. N.; Mangoni, M. L.; Merlino F.; Grieco, P. *J. Med. Chem.* **2021**, 64, 11675-11694.
4. Bellavita, R.; Leone, L.; Maione, A.; Falcigno, L.; D'Auria, G.; Merlino, F.; Grieco, P.; Nastri, F.; Galdiero, E.; Lombardi, A.; Galdiero, S.; Falanga A. *Dalton Trans. just accepted.*

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STEREOSTRUCTURAL DETERMINATION OF A NEW CYANOCHELIN FROM THE CYANOBACTERIA *LEPTOLYNGBYA*

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The marine environment hosts a huge number of micro- and macro-organisms, which are capable of producing and/or accumulating secondary metabolites and represent an incredible bioresource of biologically active and structurally unique natural products (NPs). The reasons for this complex multiplicity of secondary metabolites can be traced to a number of additional activities in which they are involved, such as intra- and inter-species communication, attack and defence mechanisms and adaptation to extreme environmental conditions, such as confined spaces, high ion concentrations and low food availability, which cause existing organisms to develop survival and defence mechanisms.[1]

In this respect, our attention is paid to siderophore from cyanobacteria.

Cyanobacteria depend on great amounts of iron as an essential cofactor for basic metabolic processes such as photosynthesis, respiration or nitrogen fixation and have a greater need for iron than non-photosynthetic organisms.

For this reason, they have developed many strategies to survive in limited iron conditions, such as the production of siderophores, which are compounds, secreted by microorganisms and plants, with low molecular weight (400-1000 kDa) considered the strongest chelators of Fe^{3+}.[2]

Recent genome sequencing techniques and the detection of multiple silent gene clusters offer an excellent starting point for the identification of novel siderophores.3

In particular, if iron-poor culture medium is used, it is possible to activate some silent gene clusters that may code for the production of unknown siderophores.

Using bioinformatic analysis and genome mining, additional gene clusters sets, presumably encoding for cyachelins biosynthesis, were identified.

In collaboration with the research group of the Institute of Microbiology, in Třeboň, Czech Republic, a new cyanochelin from cyanobacterium *Leptolyngbya sp.* was identified and isolated.

From the isolated cyanochelin sample, the stereostructural elucidation was studied through the combined use of mass spectrometry (MS) and nuclear magnetic resonance (NMR) techniques, mono- and bi-dimensional both homo- and heteronuclear, but also through the use of degradation and derivatization methods (such as the Marfey method).

REFERENCES:

1. Rinehart KL. Secondary metabolites from marine organisms. *Ciba Found Symp.* **1992**,171, 236-49.
2. Årstøl E., Hohmann-Marriott MF. Cyanobacterial siderophores—physiology, structure, biosynthesis, and applications. *Mar Drugs* **2019**, 17, 281.
3. Galica, T., Borbone, N., Mareš, J., Kust, A., Caso, A., Esposito, G., ... & Hrouzek, P. Cyanochelins, an overlooked class of widely distributed cyanobacterial siderophores, discovered by silent gene cluster awakening. *Applied and Environmental Microbiology*, **2021**, *87*, 17.

A Four-Step Platform to Optimize Growth Conditions for High-Yield Production of Siderophores in Cyanobacteria

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Siderophores are iron-chelating molecules that are produced by the cyanobacteria *Anabaena flos aquae* in response to an iron deficiency and under specific environmental conditions. These iron-chelating molecules, on account of their interesting environmental and clinical applications, have recently gained the interest of the pharmaceutical industry. Unfortunately, the yields of siderophore recovery from in vitro generating cyanobacterial cultures are relatively poor and, the majority of the time, only approach analytical values. Here, we suggest a four-step experimental workflow for the quick and low-cost discovery and optimization of growth factors that affect *Anabaena flos aquae*'s siderophore synthesis at the transcriptional level. The four-steps pipeline consists of: (1) identification of the promoter region of the operon of interest in the genome of *Anabaena flos aquae*; (2) cloning of the promoter in a recombinant DNA vector, upstream the cDNA coding for the Green Fluorescent Protein (GFP) followed by its stable transformation in *Escherichia Coli*; (3) identification of the environmental parameters affecting expression of the gene in *Escherichia coli* and their application to the cultivation of the *Anabaena* strain; (4) identification of siderophores by the combined use of high-resolution tandem mass spectrometry and molecular networking. This interdisciplinary, sustainable, and environmentally friendly pipeline is capable of being automated and is almost certainly applicable to any cyanobacteria, or more generally, to any microbes.

Keywords: schizokinen; synechobactin; Anabaena flos aquae; biosynthesis of marine drugs; cyanobacteria; iron-chelating molecules; natural products; dereplication strategy; molecular network.

REFERENCES:

1. Kundu K, Teta R, Esposito G, Stornaiuolo M, Costantino V. *Metabolites*. **2023,** 13, 154.
2. Teta R, Esposito G, Kundu K, Stornaiuolo M, Scarpato S, Pollio A, Costantino V. *Marine Drugs*. **2022,** 20, 256.

Società Chimica Italiana
Gruppo Interdivisionale Biotecnologie

WORKSHOP: *I chimici per le biotecnologie*

Napoli, 27 Febbraio 2023

The biosynthetic potential of cyanobacteria

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Cyanobacteria of marine origin are one of the sources of greatest interest both for their application in the drug discovery implementation process in the development of innovative and sustainable pharmacological lead compounds and for their function as bio-indicators of environmental health status due to their physiological adaptability that allows them to colonize different habitats and develop specific physiological mechanisms that enable them to adapt to different environmental stressors.

The monitoring of cyanobacteria through the analysis of water samples collected mainly along the coasts of the Campania region and sampled periodically under standard and bloom conditions thanks to the Fast Detection Strategy (FDS) protocol is a way to understand the biosynthetic potential of cyanobacteria to find new bioactive natural products using an innovative approach focused on cultivating cyanobacterial strains under different conditions according to the OSMAC (One Strain, Many Compounds) strategy.[1]

The cyanobacteria strains present in the samples are pre-identified by metagenomic analysis as the sequencing of the 16 S rRNA region to define the taxonomy of the cyanobacteria present in the collected samples.

The OSMAC (One Strain, Many Compounds) strategy is an innovative approach based on altering certain cultivation parameters such as nutrient content, metal ion content, degree of aeration, and temperature to activate clusters of silent biosynthetic genes not activated under normal conditions.[2] Triggering expression of these “silent” clusters could result in unlocking the chemical diversity of marine-derived cyanobacteria, allowing the discovery of novel molecules of both medical and biotechnological interest.[3]

The OSMAC approach thus offers the opportunity to allow the expression of genes that are not expressed under the environmental conditions in which cyanobacteria live and thus to be able to induce the production of secondary metabolites that are not present under normal conditions and are not detectable by analysis from bacterial extracts from direct sampling alone. Metabolomic expression of cyanobacteria is subsequently verified with dereplication studies of extracts derived both from sampling and laboratory cultures by combined liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) analysis using the molecular networking technique that allows rapid identification of known metabolites and new analogs from the extracts by highlighting the diversity of the pool of secondary metabolites produced by cyanobacteria at time of sampling and after cultivation under different conditions.

REFERENCES:

1. Wei, H. *et al.* OSMAC (one strain many compounds) approach in the research of microbial metabolites--a review. *europepmc.org*.
2. Galica, T. *et al.* Cyanochelins, an Overlooked Class of Widely Distributed Cyanobacterial Siderophores, Discovered by Silent Gene Cluster Awakening. *Appl Environ Microbiol* 87, 1–13 (2021).
3. Romano, S.; Jackson, S.A.; Patry, S.; Dobson, A.D.W. Extending the “One Strain Many Compounds” (OSMAC) Principle to Marine Microorganisms. *Mar. Drugs* 2018, *16*, 244.

Biocatalytic preparation of tryptamine derivatives as novel antimicrobial agents

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Antimicrobial resistance (AMR) is becoming a critical issue for public health and agrifood sector. Regarding the latter, different phytopathogens cause huge crop losses, heavily affecting annual harvests. For instance, the fungus *Pyricularia oryzae*, the etiological agent of rice blast, is responsible for severe yield reductions which would feed more than 60 million people every year[1]. Plants counteract biotic infections by producing phytoalexins, secondary metabolites often endowed with antimicrobial activity.

Inspired by N-trans-cinnamoyltryptamine, a natural phenylamide found in *Oryza sativae*[2], we designed and synthesised a small collection of 24 indole derivatives (F**ig.1**), using *Mycobacterium smegmatis* acyltransferase (**MsAcT**)[3] as biocatalyst to perform amidation of benzoic and cinnamic acids with tryptamine derivatives. Acids were first converted *via* Pd-catalysed Heck-type vinylation reaction[4] into the corresponding vinyl esters, the most efficient acyl donors for the MsAcT-mediated condensation. The catalysis has been optimised in order to reduce the environmental burden and to increase the sustainability of the entire process. Indeed, tetramethyloxolane (TMO) [5] a greener alternative to toluene has been employed for all the bioconversions, giving higher yields compared to other solvents. All the products have been evaluated for their activity against a panel of selected Gram-negative and Gram-positive bacteria, as well as on seven phytopathogenic fungi showing promising activity against some strains. Our results pave the way for novel nature-inspired products as valuable alternatives to currently available pesticides.

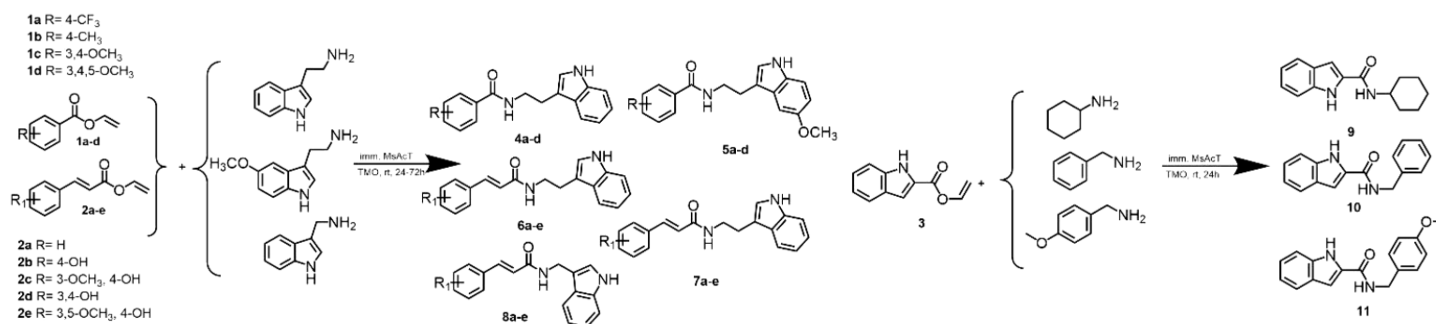

Figure.1 Tryptamines derivatives obtained

REFERENCES:

1. Pennisi, E et al. Science. 2010, 327, 804.
2. Park, HL et al. Molecules. 2014, 19, 18139.
3. Cannazza et al.j.biotechadv. 2022, 59, 107985.
4. Kadidae, LO et al. Asian J Chem. 2018, 30, 589.
5. Pellis, A et al.Green Chem. 2019, 21, 1686.

Società Chimica Italiana
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Applying enzyme engineering for commercial manufacturing of chemicals

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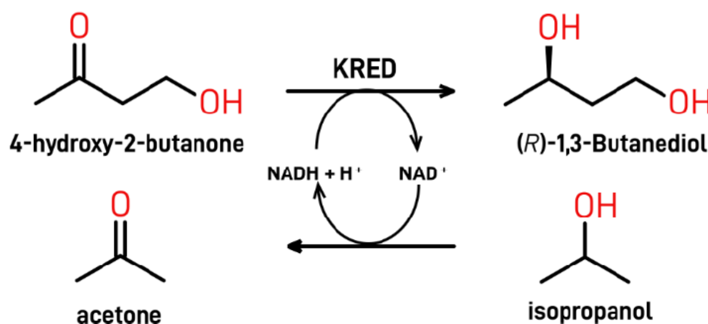
The commercialization of sustainable enzymatic and microbial catalysis technology is gaining increasing priority for the synthesis of chiral compounds from achiral precursors that require high selectivity and high substrate load. Thus, the fast and cost-effective development of novel biocatalytic processes using an integrated platform offers significant opportunities to fine chemical, pharmaceutical, food & feed, material and related industries.

As an example, a cascade using two keto reductases (KREDs) with different enantioselectivity is presented for the efficient production of (*R*)-1,3-butanediol (R-BDO) for pharma, food, and cosmetics applications on a multi ton scale. Kinetic resolution of racemic 1,3-butanediol (BDO) results in high cost and max. 50% theoretical yield of R-BDO.

We have evolved one substrate and solvent tolerant KRED for the oxidation of racemic BDO to 4-hydroxy-2-butanone driven by acetone to *iso*propanol reduction for NAD+ recycling and a highly enantioselective variant of the same KRED 1 for 4-hydroxy-2-butanone reduction to R-BDO using *iso*propanol to acetone oxidation for NADH recycling (Scheme 1). Overall, we generated an economically and environmentally friendly redox neutral cascade reaction which can re-use the *iso*propanol <=> acetone co-substrate system to drive the redox cascade on multi ton scale.



Scheme 1

1. WO2020/119740A1

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Differential sensing with arrays of de novo designed peptide assemblies

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The early diagnosis of disease increases the chances of successful treatments to reduce morbidity and mortality. Most current diagnostics and monitoring rely on the measurements of specified biomarkers. However, single biomarkers do not describe multifactorial metabolic processes and can often be very limited in their clinical effectiveness. Omics approaches, such as metabolomics, that provide collections of markers are becoming favoured over the detection of single biomarkers as they better describe multifactorial processes. Cells and tissues constantly exchange with their surrounding fluids and release a variety of metabolites into them. As a result, the biochemical state of the cells is reflected in these fluids. The most common analytical techniques used in metabolomic studies of such fluids are intensive and time-consuming. We have contributed to introducing an alternative method to assay the fluids through a biomolecular differential sensing approach that overcomes this and other limitations of traditional analytical techniques. Recognition through this approach is not achieved by a strong affinity for a specific receptor, but rather by the composite response of an analyte or mixture to an array of less-specific receptors. This is inspired by mammalian olfactive systems and how they recognise a vast number of volatile molecules with limited sets of receptors. Such differential or array-sensing approaches do not require the identification of individual components in the mixture as the receptors within the array bind to multiple analytes to different extents producing a unique signature for the analysed sample.

The research focussed on α-helical barrels (αHBs) and their supramolecular host-guest interactions. Specifically, my objective was to design and synthesize new peptide assemblies mimicking the mammalian senses of smell for differential sensing of small molecules in complex mixtures of biological samples. The selfassembling αHBs were made with altered central channels to vary their sizes, shapes and chemistries. The channels can accommodate an environment-sensitive dye that fluoresces upon binding. Thus, the dye-loaded barrels were arranged in 48-element arrays and challenged with analytes causing differential fluorophore displacement. The resulting fluorimetric fingerprints were used to train machine-learning models that relate the patterns to the analytes. This system discriminates between a range of biomolecules (fatty acids, aminoacids), drinks (green and black tea), and diagnostically relevant biological samples (sera). In fact, clinical samples from non-alcoholic steatohepatitis (NASH) and coronary artery disease (CAD) donors can be classified and predicted. As αHBs are robust and chemically diverse, the system has the potential to sense many analytes in various settings.[1]

REFERENCES:

1. Dawson, W.M. at al., *Nat Commun.* **2023**, *14,* 383.

Self-assembled peptide-based nanostructures as multivalent tools for biotechnological applications.

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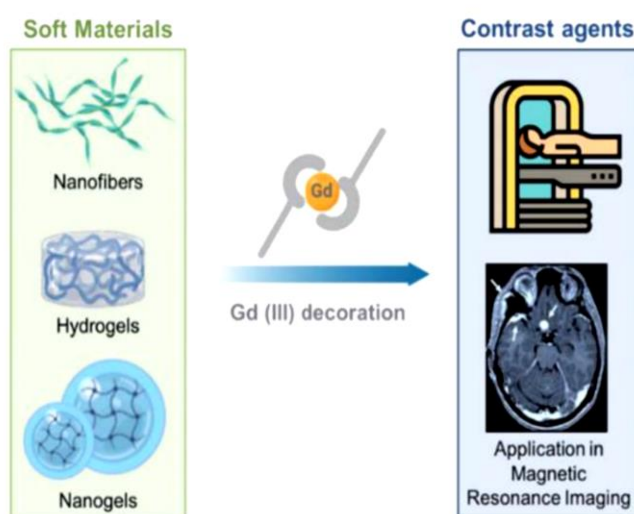
Magnetic Resonance Imaging (MRI) is one of the most powerful techniques currently used in clinic for the diagnosis of tumoral pathologies in their early stages. The quality of the images is improved by administering to patients a T1 or T2 Contrast Agents (CAs) like Gd-complexes or superparamagnetic nanoparticles (SPIONs), respectively. In the last years, novel strategies, based on the use of nanomedicine, have been proposed to enhance the CAs performances.[1] Biotechnological nanoplatforms consent to simultaneously deliver a high number of CAs in the site of interest. Moreover, the grafting or the encapsulation of CAs within the nanostructures also allow to increase the relaxivity of each CA. Many different macromolecular and supramolecular structures like dendrimers, polymers, liposomes, micelles and fibers have been proposed as innovative CAs until now.[2] Recently, novel classes of soft biomaterials such as hydrogels (HGs) and nanogels (NGs) have emerged.[3] In this context, we recently developed supramolecular structures based on the self-assembling of short and ultrashort peptide sequences.[4,5] Peptides have been proposed as building blocks for generating nanostructured aggregates thanks to their advantages such as: biocompatibility, bioavailability, chemical versatility and high synthetic accessibility. Self-assembled HGs and NGs were exploited as platforms for the vehiculation of Gd-complexes (Gd-DTPA, Gd-DOTA, Gd-AAZTA and Gd-BOPTA) or as an alternative of Chemical exchange saturation transfer (CEST)-CAs. The resulting biomaterials were characterized from the structural, mechanical and morphological point of view. The loading and the release of the CAs were optimized and the relaxometric behaviour investigated. Finally, the more promising systems were also studied both *in vitro* and *in vivo*.



REFERENCES

1. Siddique, S.; Chow, J.C.L. *Nanomaterials.* **2020**, *10*, 1700-1740.
2. 2. Diaferia, C.; Gianolio, E.; Accardo, A.; *J. Pept. Sci.* **2019**, *25*, e3157.

3.
4. Gallo, E.; Rosa, E.; Diaferia, C.; Rossi, F.; Tesauro, D.; Accardo, A. *RSC Adv.* **2020**, *10*, 27064-27080.
5. Gallo, E.; Diaferia, C.; Morelli, G.; Gianolio, E.; Accardo, A.; *Pharmaceuticals.* **2020**, *13*, 19.
6. Rosa, E.; Carniato, F.; Tei, L.; Diaferia, C.; Botta, M.; Accardo, A.; *Pharmaceuticals.* **2022**, *15*, 1572.

Development of new Antibody Drug Conjugates with antiviral activity

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Composed by a monoclonal Antibody (mAb) connected by a proper linker to a drug (called payload), Antibody Drug Conjugates (ADCs) are interesting biotechnological drugs successfully applied in cancer therapy with 12 ADCs approved by the FDA up to 2022[1]. The possibility to charge monoclonal Antibodies with not-cytotoxic drugs targeting specific cancer targets (i.e. HDAc inhibitors) [2] has been recently reported by our team, opening to the application of ADCs to different field beyond cancer. Few examples of antimicrobial ADCs have been reported by Genethec still using cytotoxic antibiotics as the payloads.[3] We here report out studies on the development of ADC as antiviral agents. Indeed, COVID19 pandemic shows to everybody the urgent need of new approaches to face viral infections and future pandemic. In this work we report our last findings in the exploration of the ADC technology in the antiviral field by using SARS-CoV2 as a model to set up all the experiments. For our purpose we are exploring the use of a mAb that have already pass late stage clinal trials for COVID-19 treatment that has been developed by Toscana Life Sciences: J08 (MAD0004J08).[4] As payload we choose Doxorubicin, an extremely toxic anticancer drug, which shows potential activity against SARS-Cov-2 proteases[5] and Niclosamide, an anthelmintic able of neutralizing endosomal pH and so viral proliferation and release.[6] The *linkers* are cleavable and non-cleavable to exploit different release mechanisms: a cleavable one, able to release the payload after physiological stimuli (i.e., low pH, protease) recently developed in our laboratories [7] and a non-cleavable one with to explore the effect of a slower release of the payload. All bioconjugates have been characterized via MALDI-TOF technology to establish DAR (drug antibody ratio).

REFERENCES:

1. Baah, S.; Laws, M. *et al. Molecules*. **2021**, *26*, 2943.
2. Cini, E.; Faltoni, V.; Petricci, E. *et al. Chem. Sci.* **2018**, *9*, 649.
3. Lehar, S.M.; Pillow, T. *et al. Nature.* **2015**, *527*,323.
4. Andreano, E.; Nicastri, E. et al. *Cell***. 2021**, 184, 1821.
5. Jamal, Q. M. S.; Alharbi, A. H. *et al. Biomol. Struct. Dyn.* **2021**, *7*, 1.
6. Gassen, N. C.; Niemeyer, D. *et al. Nat. Commun.* **2019**, *10*, 5770.
7. Migliorini, F.; Cini, E. *et al. Chem. Commun.* **2022,** 58, 10532.

Mycobacterium smegmatis acyltransferase: the big new player in biocatalysis

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The combination between biocatalysis and continuous processing has been identified as one of the foremost key techniques for sustainable manufacturing of food ingredients, APIs and fine chemicals.[1] To increase the uptake of biocatalytic processes by industry, it is essential to demonstrate the reliability of enzyme-based methodologies directly applied to the production of high value products.

A versatile and chemoselective acyltransferase from *Mycobacterium smegmatis* (MsAcT) was selected for its remarkable features: its characteristic hydrophobic tunnel leading to the active site disfavours the ingress of water, thus promoting ester formation over hydrolysis also in aqueous media.[2] A straightforward one-step biocatalyzed synthesis of different flavour esters and *N*-acetyl derivatives starting from primary alcohols/amines and a variety of acyl donors in water was accomplished in batch using the free enzyme.[3-5] Subsequently, an efficient, unique, and sustainable enzymatic platform for the multi-gram synthesis of natural aroma-compounds and melatonin-analogues was developed (Fig. 1).[6,7] The system exploits the covalent immobilization of MsAcT onto agarose beads increasing the robustness and longevity of the immobilized biocatalyst. The fully-automated process deriving from the integration between biocatalysis and flow chemistry is designed to maximize the overall yields and reduce reaction times (5 min), overcoming the limitations often associated with bioprocesses, finally bridging the gap between lab scale and industrial production.

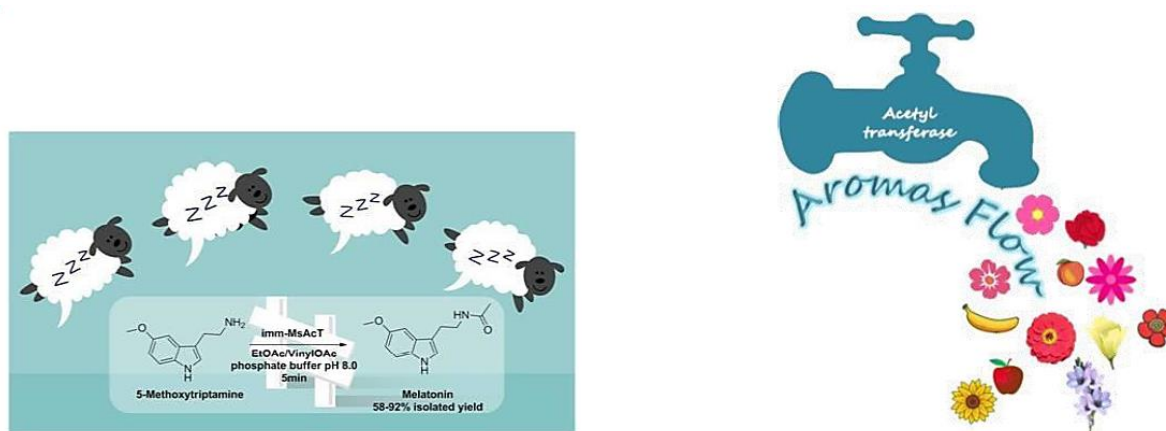

Figure 1, *Sustainable MsAcT high productivity processes*

REFERENCES:

1. Jiménez-González, C. *et al. J. Org. Process Res. Dev.* **2011**, *15*, 900.
2. Mathews, I. et al. *Biochemistry* **2007**, *46*, 8969.
3. Chiarelli Perdomo, I. *et al. J. Agric. Food Chem.* **2019**, *67*, 6517.
4. Contente, M. L. *et al. Adv. Synth. Catal*. **2018**, *360*, 4814.
5. Christodoulou, M. S. *et al.* Green Chem. **2022**, *24*, 4432.
6. Contente, M. L. *et al.* Green Chem. **2019**, *21*, 3263.
7. Contente M. L. *et al.* J. Flow Chem. **2020**, *10*, 235.

ZnO nanowire synthesis and biotechnological application

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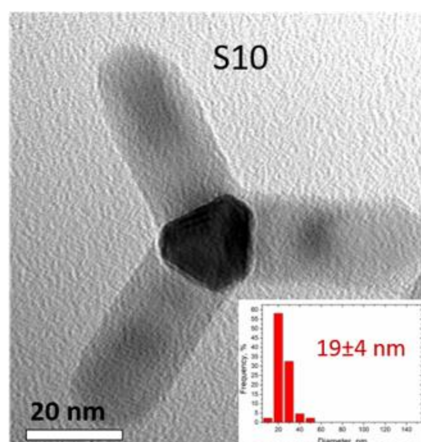
ZnO nanoparticles, as low cost and low toxic materials, have shown promising performances in biotechnological application. ZnO nanowires (NWs) are the nanoparticles with an elongated, high aspect ratio form. Focus of our work is on the feasible synthesis of ZnO NWs (or ZnO tetrapods) using high yield methods and their excellent performance in sensing. We use ZnO NW based sensor platform, which involves chemoresistive sensing mechanism, low temperature preparation methods, therefore virtually any surface could be covered with a controllable thickness and morphology of ZnO NWs implying a wide range of application possibilities. We also established a correlation between the pore size in different ZnO nanostructures because of packing and their electrochemical properties. We expect that the detailed analysis of ZnO NWs will be advantageous for future electrochemical, biosensing and biotechnological applications of these materials.



Combining molecular recognition studies, natural and synthetic compounds to develop molecular tools to modulate biological systems.

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Biotechnology requires the integration of natural and engineering sciences to exploit living organisms, cells, parts thereof and molecular analogues to obtain products and services.

As chemists and biotechnologists, we mainly deal with the discovery of molecules capable of modulating biological systems, with the aim of contributing to the development of new molecular tools for prevention and treatment of diseases and/or for their diagnosis.

This communication will be focused on the experimental workflow through which we combine the chemical characterization and screening of natural extracts by metabolomics and molecular recognition studies with organic synthesis to obtain bioactive synthetic variants of natural compounds and/or multivalent molecules.

This workflow implies a multidisciplinary approach integrating advanced analytical techniques, organic synthesis, biochemical, biophysical, and biological assays, expression and purification of heterologous proteins and genetic engineering of cell lines.

Eco-friendly, biologically-based techniques for cleaning water

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The scarcity of clean drinking water, particularly in Third World nations, is currently one of the major worldwide issues. A possible approach to solving this issue might be to create water filtration systems that are simple to use, affordable, and ecologically friendly. Antimicrobial peptides (AMPs), which have excellent activity against target pathogens and are quickly and fully biodegradable, are intriguing options for water purification. AMPs are characterized by three important properties: (I) small dimensions, (II) highly cationic character, and (III) tendency to adopt amphipathic structures. These properties explain the ability of AMPs to bind negatively charged bacterial membranes and destroy the cells by several possible mechanisms. The aim of this project is to develop water purification filters (Figure 1) based on the immobilization of antimicrobial peptides on polymer supports. The antimicrobial sequence NH2-K-R-W-W-R-W-R-COOH was selected to be bound to the polymer; for this purpose, the synthetic strategy of "grafting through" was chosen. The resulting compound was analyzed physicochemically by various techniques, including differential scanning calorimetry (DSC), X-ray diffraction, UV-vis, and NMR spectroscopy. Currently, biological assays are being performed with the lone peptide and will be performed on the hybrid compound to verify that it retains its bactericidal properties after immobilization. At the same time, various machine learning algorithms, such as Python and Amplify, are being used to obtain new promising sequences. The resulting peptide-nylon polymers will subsequently be used to produce water filter membranes.

Figure 1: Antimicrobial filter for water purification.

REFERENCES:

1. S.Jebors, C.Pinese, T.Montheil, A.Bethry, S.Verquin, L.Plais, M.Moulin, C.Dupont, X.Garric, A.Mehdi, J.Martinez, G.Subra, European Polymer Journal (2020), Vol.135, 109886
2. S.Clark, T.A.Jowitt, L.K.Harris, C-G-Knight, C.B.Dobson, Communication biology (2021), 605

Myxinidin-derived peptide efficacious in fighting cystic fibrosis emerging pathogens

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Cystic Fibrosis is an autosomal recessive disease characterized by mutations in the CF transmembrane conductance regulator gene (CFTR), involved in the production of viscous mucus that promotes bacterial growth and development of chronic infections[1]. To overcome the use of antibiotics and reduce the risk of bacterial resistance, antimicrobial peptides (AMPs) have proven to be a promising alternative for their antimicrobial, anti-inflammatory, and immunomodulatory activities.[2] Herein, we designed, synthesized, and characterized a more serum-stable version of the peptide WMR (WMR-4), which derived from the natural peptide Myxinidin isolated from a marine organism. WMR-4 demonstrated able to eradicate single and dual- species biofilms of *C. albicans*, *S. maltophilia*, *A. xylosoxidans*, which are three microorganisms involved in CF infections.[3] The biological results confirmed a better antimicrobial activity than the native peptide WMR and it resulted to be not toxic in a *in vivo* model using *Galleria mellonella* larvae. Fluorescence measurements and circular dichroism (CD) spectroscopy were performed to establish the hypothetical mechanism of action. The biophysical analysis on LUVs mimicking Gram-negative membranes displayed a hypothetical capacity of WMR-4 to insert into lipo-polysaccharide (LPS) and in bacterial and fungal membranes. When the peptide interacts with the model of fungal and bacterial membranes, we hypothesized a membrane disruption through the carpet-like mechanism. Our results provide that antimicrobial peptides and WMR-4 can be used for therapeutic applications in CF patients suffering from bacterial infections.

REFERENCES:

1. Allan, K.M.; Farrow, N.; Donnelley, M.; Jaffe, A.; Waters, S.A. Treatment of cystic fibrosis: from gene- to cell-based therapies. *Front. Pharmacol.* **2021**, 12:639475.
2. Zhang, L.; Parente, J.; Harris, S.M.; Woods, D.E.; Hancock, R.E.; Falla, T.J. Antimicrobial peptide therapeutics for cystic fibrosis. *Antimicrob. Agents Chemother.* **2005**, 49, 2921–2927.
3. Bellavita, R.; Maione, A.; Braccia, S.; Sinoca, M.; Galdiero, S.; Galdiero, E.; Falanga, A. Myxinidin-derived peptide against biofilms caused by cystic fibrosis emerging pathogens. *Int. J. Mol. Sci.* **2023**, just accepted.

Nanoformulations based on clay minerals for local treatment of solid tumors

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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, doseresponse 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 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 future local treatment of bladder cancer.

REFERENCES:

1. Massaro M.; Noto R., Riela S. *Molecules*, **2020**, *25*, 4863.
2. Massaro M.; Licandro E.; Cauteruccio S.; Lazzara G.; Liotta L. F.; Notarbartolo M.; Raymo F. M.; Sánchez-Espejo R.; Viseras-Iborra C.; Riela S. *J. Coll. Int. Sci.* **2022**, *620*, 221-233.
3. Massaro M.; Notarbartolo M.; Raymo F.; Cavallaro G.; Lazzara G.; Mazza M. M. A.; Viseras- Iborra C.; Riela S. *ACS Appl. Nano Mater.* **2022**, *5, 10*, 13729–13736.
4. Massaro M.; Poma P.; Cavallaro G.; Garcìa-Villén F.; Lazzara G.; Notarbartolo M.; Muratore N.; Sànchez-Espejo R.; Viseras-Iborra C.; Riela S. *Coll. Surf. B* **2022**, *213*, 112385.

Towards New Exogenous Antioxidant Supplements organo-Se-based

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Even though oxidative stress (OS), a disequilibrium redox state, is a major risk factors for the development of several medical illnesses, such as cardiovascular disease, age-related neurodegenerative diseases, and several types of cancer, epidemiological studies have shown that eating foods high in antioxidants or using exogenous antioxidant supplementation may help to lower this risk. [1]

Nutraceuticals are of particular interest; indeed, they might be used to prevent or mitigate OS by providing other health advantages in addition to nutrition.[2] Within this framework, our research is focused on Selenium (Se) and polyphenols. The former is one of the trace elements involved in immunological system, growth, healthy muscular activity, reproductive organs; it decreases the toxicity of some elements such as mercury responses and its deficiency is linked to immune, cardiac, muscular, bone, and other diseases. [3] Polyphenols are also acknowledged to have health-promoting properties, although their limited bioavailability is frequently blamed. In assessing their real potential as an approach to combating diseases, it has been shown that glycosylation affects the chemical, biological, and physical characteristics of polyphenols, allowing for greater absorption in the small intestine. [4]

Thus, the synthesis of organo-Se-compounds with a sugar-type structure coupled with (poly)phenols via Mitsunobu reactions aims to provide novel molecules that could overcome limited bioavailability while also providing a synergistic antioxidant effect. [5] DPPH and ABTS antiradical tests were used to evaluate the bioactivity of selenoglycoconjugates. Moreover, the effects on cell proliferation were investigated towards Caco-2 intestinal epithelial cells, SHSY5Y neuroblastoma cells, and HaCaT keratinocytes. The phenol moiety has a significant impact on both antiradical activity and mitochondrial redox activity. The cytotoxic effects of glycoconjugates, particularly at the highest tested doses, were lower than those of unconjugated phenolic compounds, highlighting the moderating influence of selenosugar.

REFERENCES:

1. Bollati, C., Marzorati, S., Cecchi, L., Bartolomei, M., Li, J., Bellumori, M., d'Adduzio, L., Verotta, L., Piazza, L., Arnoldi, A., Mulinacci, N.; Lammi, C. (2023). *Antioxid*. **2023,**(1), 144.
2. Anand, S.; Bharadvaja, N. *Rev Bras Farmacogn* **2022,** *22*, 211-220.
3. Tóth, R.; Csapó, *Acta Aliment*. **2018**, 128-144
4. Almeida, S.; Alves, M. G.; Sousa, M.; Oliveira, P. F.; Silva, B. M. *Neurotox. Res.* **2016***, 30*(3), 345–366.
5. Serpico, L.; De Nisco, M.; Cermola, F.; Manfra, M.; Pedatella, S. *Molecules* **2021**, *26*, 2541.

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Exploring Different Synthetic Strategies to Develop Halloysite-CarbonDots Nanomaterials

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Halloysite is a natural clay mineral with a predominantly hollow tubular structure (HNTs). 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] It presents an external surface composed by siloxane groups and an inner lumen constituted by aluminum hydroxide. 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 first 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 second one where free carbon dots previously synthetized were linked on the external surface of modified HNTs. The nanomaterials obtained were characterized by several techniques (FT-IR, TGA, XPS, DLS, Z-potential and TEM) and their photoluminescence, antioxidant and cellular uptake properties were investigated as well.

REFERENCES:

1. Massaro M.; Noto. R.; Riela. S. *Molecules* **2020**, *25*, 4863.
2. Riela. S.; Barattucci A.; Barreca D.; Campagna S.; Cavallaro G.; Lazzara G.; Massaro M.; Pizzolanti G.; Salerno
3. T. M. G.; Bonaccorsi P.; Puntoriero F. *Dyes pigm.* **2021**, *187*, 109094.
4. Massaro M.; Colletti G. C.; Guernelli S.; Lazzara G.; Liu M.; Nicostra G.; Noto R.; Parisi F.; Pibiri I.; Spinella
5. C.; Riela S. *J. Mater. Chem. C* **2018**, *6*, 7377-7384.

Probing the Role of Chirality in the Ca^{2+} Mobilizing Properties of New cADPR Linear Precursors

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Cyclic adenosine diphosphate ribose (cADPR, **1**) is a cyclic nucleotide involved in the Ca^{2+} homeostasis. In its structure, the northern ribose, bonded to adenosine through an N1 glycosidic bond, is connected to the southern ribose through a pyrophosphate bridge. Due to the chemical instability at the N1 glycosidic bond, new bioactive cADPR derivatives have been synthesized. One of the most interesting analogues is the cyclic inosine diphosphate ribose (cIDPR), in which adenosine is replaced by hypoxanthine. In the last few years, we have produced new flexible cIDPR analogues, where the northern ribose has been replaced by alkyl chains. With the aim to obtain the closest flexible cIDPR analogue, we have attached to the inosine N1 position a 2′′,3′′-dihydroxypentyl chain, possessing the two OH groups in a ribose-like fashion, and synthesized the new cyclic analogue **2**.[1] Interestingly, the linear precursor **3** displayed a higher potency in increasing $[Ca^{2+}]_i$ than the cyclic compound **2** in primary cortical neurons. The compound **3** was obtained as a 1:1 mixture of two diastereomers, as its inosine precursor **4** was reacted with the racemic tosylate **5**. To probe the role of the chirality of 2′′ and 3′′ carbon atoms in the Ca^{2+} releasing properties of the compound **3**, herein we report on the synthesis of the two diastereomers (2′′*S*,3′′*R*)-**3** and (2′′*R*,3′′*S*)-**3** (Figure 1).

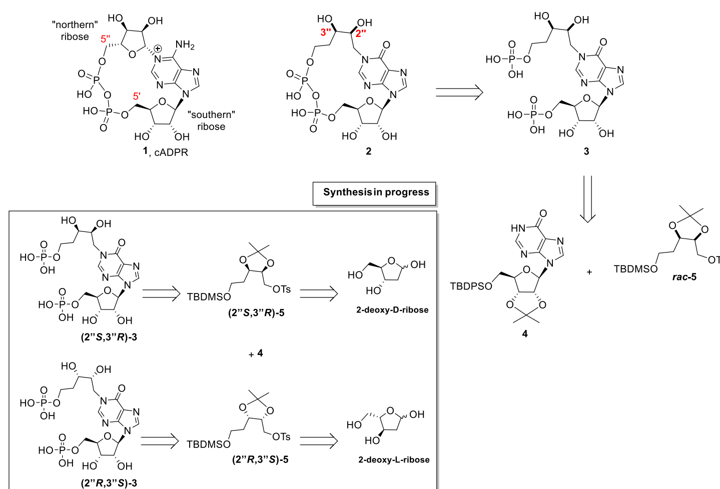

***Figure 1**: Proposed synthesis of the novel cADPR linear precursors (2′′S,3′′R)-**3** and (2′′R,3′′S)-**3**.*

REFERENCES:

1. S. D'Errico *et al*., *Bioorg. Chem*. **2021**, *117*, 105401.

Protein-peptide interaction on living cells by NMR

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The interaction of peptides with biological membranes is central to a number of biological processes, such as the insertion and folding of peptides in membranes, the rupturing of membranes by toxins and the membrane-mediated mechanism of peptide-protein interaction. To target membrane-bound receptors, several homonuclear NMR spectroscopic experiments have been extensively used in their physiological environment [1,2], detecting binding events and providing information on the bound conformation of the ligands. Herein, we show how NMR methodologies has been used to investigate binding events at the cell surface between designed VEGF mimetic peptides and membrane-bound proteins [3,4].

REFERENCES:

1. Ito, Y.; Selenko, S. *Curr. Opin. Struct. Biol.* **2010**, *20.*
2. Potenza, D; Vasile, F.; Belvisi, L.; Civera, M.; Araldi, E.M.V.; *ChemBioChem* **2011**, 12.
3. Diana, D.; Riussomanno, A.; De Rosa, L.; Di Stasi, R.; Di Gaetano, S.; Romanelli, A.; Fattorusso, R.; D'Andrea. L.D. *Chem. Eur. J.* **2015**, 21.
4. Di Stasi, R.; Diana, D.; Capasso, D.; Di Gaetano, S.; de Rosa, L.; Celentano, V.; Isernia, C.; Fattorusso, R.; D'Andrea. L.D. *Chem. Eur. J.* **2018**, 61.

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Carbon dots as a versatile tool to monitor insulin aggregation.

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Protein folding is crucial for proper cellular functions, and misfolding phenomenon is associated with the arising of many neurodegenerative diseases.
The early stages are noteworthy for protein aggregation since they play a pivotal role in the onset and progression of the disease, and also, their detection can be decisive for the preventive analysis of diseases.
Although the identification of early stages is of paramount importance, traditional techniques commonly used to monitor protein aggregation, such as ThT fluorescence, Circular Dichroism and Dynamic Light Scattering are blind to the initial stages of the process being able to detect only β-sheet formation.
Our previous work has demonstrated the ability to modulate the fluorescence properties of carbon dots through covalent chemical modification of their surface, allowing the discrimination of isobaric peptides[1] where the identification by other techniques had failed.
In this contribution, we have exploited these fluorescence variations of carbon dots to follow the insulin aggregation process as a function of time. Our results unveil the ability of carbon dots to distinguish distinctive oligomeric stages[2] performed by insulin before it turns into a β-sheet formation, instead of the above-mentioned techniques which can only uncover the formation of fibrils.
The chemical neighbourhood sensitiveness shown by the fluorescence properties of carbon dots joined with their increasingly widespread application in biotechnology[3], make them a powerful tool for the development of new strategies for preventing misfolding and slowing the progression of neurodegenerative diseases.

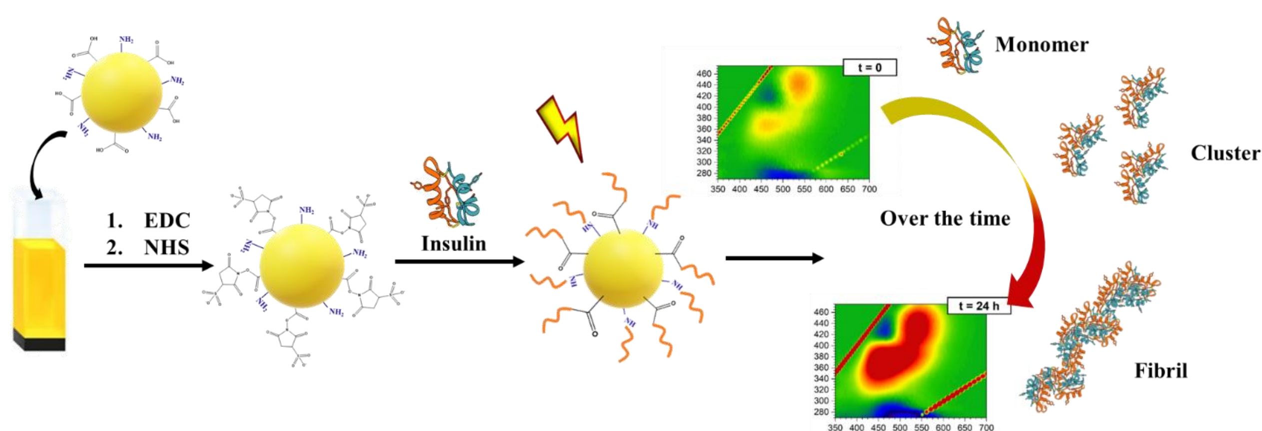

Figure 1. Schematic illustration of CDs as a biotechnological tool to monitor insulin aggregation.

REFERENCE:

1. Distefano, A.; Calì, F; Gaeta, M; Tuccitto, N, et al. J Colloid Interface Sci. **2022**, *625,* 405-414.
2. Lee, CC; Nayak, A; Sethuraman, A; Belfort, G; McRae, GJ. Biophys. J. **2007**, *92,* 3448-3458.
3. Molaei, MJ. Talanta. **2019**, *196,* 456-478.

Exploring the potential of catalytic amyloid fibrils for the development of sustainable functional materials

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Amyloid aggregates, historically implicated in human diseases, have emerged as a promising class of sustainable biomaterials for both in vitro and in vivo application. Amyloids consist of peptide monomers that self-assemble into nanofibrils with b-type structures.[1] Their unique properties at the nanoscale include high mechanical strength, biocompatibility, biodegradability as well as thermal and chemical stability. Furthermore, amyloid assemblies can be orthogonally functionalized with a wide range of biomolecules, such as enzymes and large proteins.[2] Discovery of such amyloids, referred to as multifunctional amyloids, highlights their possible use in designing novel nanostructure materials acting as biosensors, drug delivery systems and catalysts for complex syntheses.

Figure 1: Schematic representation of the conjugation strategy.

In this context, we are developing nanofibrils based on natural and de novo designed peptides as a “proof of principle” strategy for the construction of functional nanomaterials. TTR$_{105-115}$ peptide, a fragment of human transthyretin, has been selected as the self-assembling sequence. The resulting fibrils have been covalently functionalized with a synthetic mini-peroxidase (FeMC6*a) using the strain-promoted azide-alkyne cycloaddition click chemistry (SPAAC, **Figure 1**). The resulting bionanoconjugate has been thoroughly characterized by means of several techniques, such as mass spectrometry and transmission electron microscopy. The catalytic properties of the resulting nanomaterial have been evaluated using model oxidation reactions. This work aims at showing that this nanomaterial retains the catalytic properties of the artificial metalloprotein FeMC6*a, while preserving its amyloid nanostructure. This strategy will open new opportunities for the development of innovative materials with applications in biosensing and biocatalysis.

REFERENCE:

1. Hjorth, R.; van Hove, L.; Wickson, F. *Nanotoxicology* **2017**, *11* (3), 305–312.
2. Al-Halifa, S.; Babych, M.; Zottig, X.; Archambault, D.; Bourgault, S. *Peptide Science* **2019**, *111* (1), e24095.

Naphthalenediimides-based emissive probes for nucleic acid detection

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Naphthalenediimides (NDIs) are among the most versatile fluorophores explored throughout the last century.[1] The first synthetic report on core-substituted NDIs dates to 1937 while more accessible synthetic routes to modulate the NDI features and photophysical properties go back to the 2000s.[2,3] NDIs are nowadays employed in a wide range of applications such as supramolecular chemistry, photovoltaics, ion-channels, catalysis through anion−π interactions, organic electronics and biological chemistry.[1] Biologically relevant applications of NDIs are related to nucleic acids, lipid bilayer membranes and biosensors for cells imaging.[4-6] Since 70s NDIs have been proposed as DNA intercalators due to their small size and flat profile which make them the suitable candidates for the accommodation between the base pairs in DNA duplex.[4]

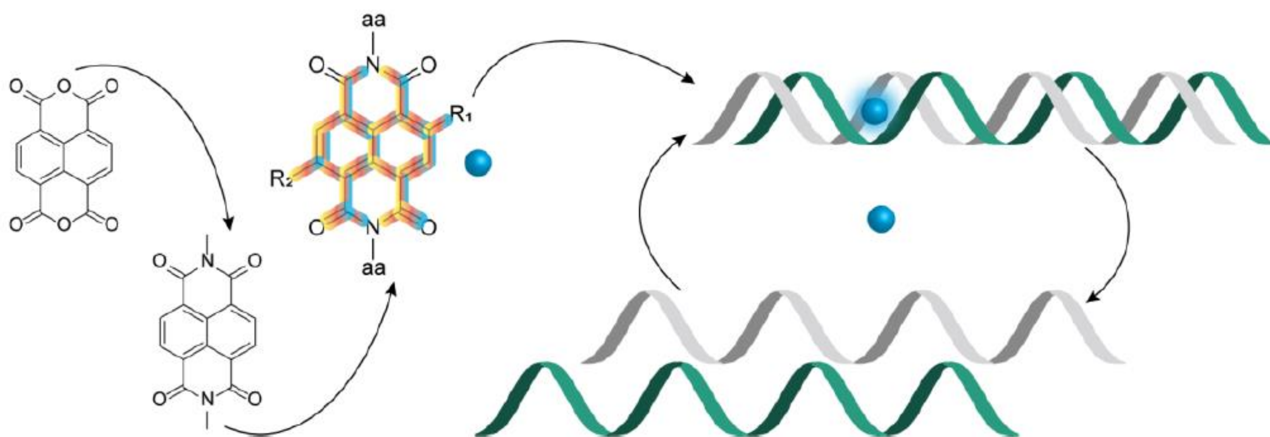

Figure 1. Emissive DNA intercalating NDI.

Here we report the preliminary investigation of NDIs decorated with various substituents at the imide side which, to the best of our knowledge, are the first example of fluorogenic NDIs able to intercalate into double stranded DNA. Preliminary results indicate that the amphiphilic balance on the NDI molecular structure is crucial to prompt an efficient intercalation. Too hydrophobic or too hydrophilic fluorophores are characterized by either very low or invariant emission signals in physiological buffer upon titration with DNA. The NDI ability to intercalate, the fluorescence turns on, and the DNA sequences will be analyzed in relationship to the NDI core functionalization moieties as well as to the substituent nature on the imide edges to fish out the most suitable candidate for the visualization of the nucleic acids in cells.

REFERENCE:

1. Bhosale, S. V.; Al Kobaisi, M.; Jadhav, R. W.; Morajkar, P. P. *Chem. Soc. Rev.* **2021**, *50*, 9845-9998.
2. Vollmann, H.; Becker, H.; Corell, M.; Streeck, H. *Liebigs Ann*. **1937**, *531*, 1-159.
3. Fin, A.; Petkova, I.; Alonso Doval, D.; Sakai, N.; Vauthey, E.; Matile, S. *Org. Biomol. Chem.* **2011**, *9*, 8246.
4. Gabbay, E. J.; DeStefano, R.; Baxter, C. S. *Biochem. Biophys. Res. Commun.* **1973**, *51*, 1083.
5. Alonso Doval, D.; Fin, A.; Takahashi-Umebayashi, M.; Riezman, H.; Roux, A.; Sakai, N.; Matile, S. *Org. Biomol. Chem.* **2012**, *10*, 6087.
6. Khan, G. S.; Shah, A.; Zia-Ur-Rehman; Barker, D. *J. Photochem. Photobiol. B Biol.* **2012***, 115,* 105.

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Hybrid peptide-based hydrogels as innovative platforms for biotechnological applications

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Peptide based materials like nanofibers, nanotubes, nanospheres and hydrogels have recently attracted great interests as innovative platforms in nanomedicine. Hydrogels are three-dimensional networks able to retain large amount of water or other biological fluids. According to their mechanical and morphological features, hydrogels have been proposed for many different biological and pharmaceutical applications including drug delivery and tissue engineering [1,2]. Due to its capability to gel under physiological conditions of pH and ionic strength, Fmoc-FF (Nα-fluorenylmethoxycarbonyl-diphenylalanine, Fig. 1) is one of the most studied low molecular-weight hydrogelators [3]. The addition of other components (like natural or synthetic polymers, peptides, polysaccharides or organic molecules) to the Fmoc-FF has been found to improve and tune the physiochemical features of the final material [4]. Here we report the formulation of hybrid hydrogels in which Fmoc-FF building block has been combined at different molar ratios with four novel synthetic three-peptides (Fmoc-FFK, Fmoc-FFC, Fmoc-FFS and Fmoc-FFT, Fig. 1). These peptides keep the Fmoc-FF motif and present an additional residue of Lys, Cys, Ser or Thr at their N-terminus. The supramolecular behaviour of the three-peptides alone or in combination with the Fmoc-FF were investigated using a set of techniques (FTIR, CD, fluorescence, and optical microscopy). All the resulting materials present on their surface a functional entity (thiol, amine or alcoholic group) that can be used for the selective derivatization of the matrix with bioactive molecules of interest.

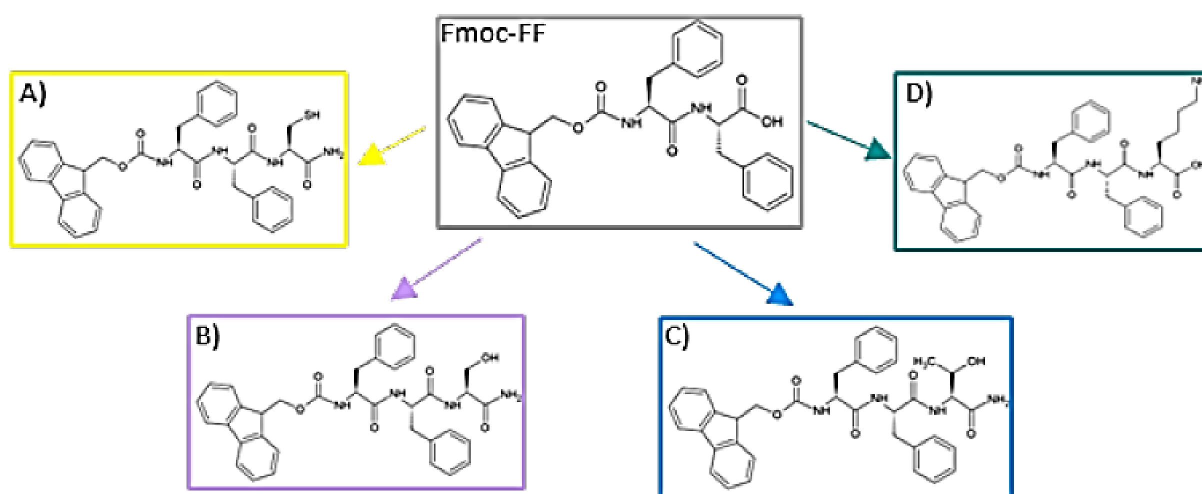

Figure 1. *Schematic representation of peptide building blocks: Fmoc-FF, Fmoc-FFC (A), Fmoc-FFS (B), Fmoc-FFT (C), Fmoc-FFK (D). Amino acids are indicated according to one code letter.*

REFERENCES:

1. *Diaferia, Carlo; Ghosh, Moumita; Sibillano, Teresa; Gallo, Enrico; Stornaiuolo, Mariano; Giannini, Cinzia; Morelli, Giancarlo; Adler-Abramovich, Lihi; Accardo, Antonella (**2019**). Soft Matter, 15(3), 487–496.*
2. *Oliveira, Carlos B. P., Valéria Gomes, Paula M. T. Ferreira, José A. Martins, and Peter J. Jervis. (**2022**). Gels.; 8(11):706.*
3. *Rosa, E., Diaferia, C., Gianolio, E., Sibillano, T., Gallo, E., Smaldone, G., Stornaiuolo, M., Giannini, C., Morelli, G., & Accardo, A. (**2022**). Macromolecular bioscience, 22(7), e2200128.*
4. *Makam, P., & Gazit, E. (**2018**). Chemical Society Reviews, 47(10), 3406–3420.*

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Use of Peptide Nucleic Acids as potential antigenic approach for genetic modulation

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Synthetic oligonucleotides are tools used to study the interaction with nucleic acids in the light of the many conformations that DNA can assume, which vary in the number and orientation of strands, the type of hydrogen bonds and the structural differences that occur due to variations in the binding angles of the sugar phosphate backbone. Given the large number of monomers that make up DNA, the analysis of such a large molecule is complex. Therefore, it is easier to analyze smaller molecules such as oligonucleotides, which mimic the structural motif of nucleic acids. Using this system, it is possible to study non-canonical DNA structures (duplex, triplex, quadruplex, hairpin and others). Among synthetic oligonucleotides, oligo-PNAs belong to the third generation of modified oligonucleotides, as they are powerful biomolecular tools with several promising applications in biotechnology: as probes for novel biosensing platforms, as genetic modulators involved in antigene, antisense and anti-miRNA strategies. As far as genetic modulation is concerned, special attention is paid to CLL, as it is known that several proteins are involved in this disease and the proof of concept is the modulation of the concentration of specific pathological proteins [1]. Here we report the identification of a new potential molecular target identified within the promoter of an investigated gene, as well as the setting up of a novel synthesis strategy aimed at obtaining PNA molecules potentially able to act as gene modulators. At the molecular level, the strategy involves the PNA-mediated antigenic approach, known as *triplex invasion*, which leads to the formation of PNA/DNA_2 complexes (Fig. 1) [2,3].

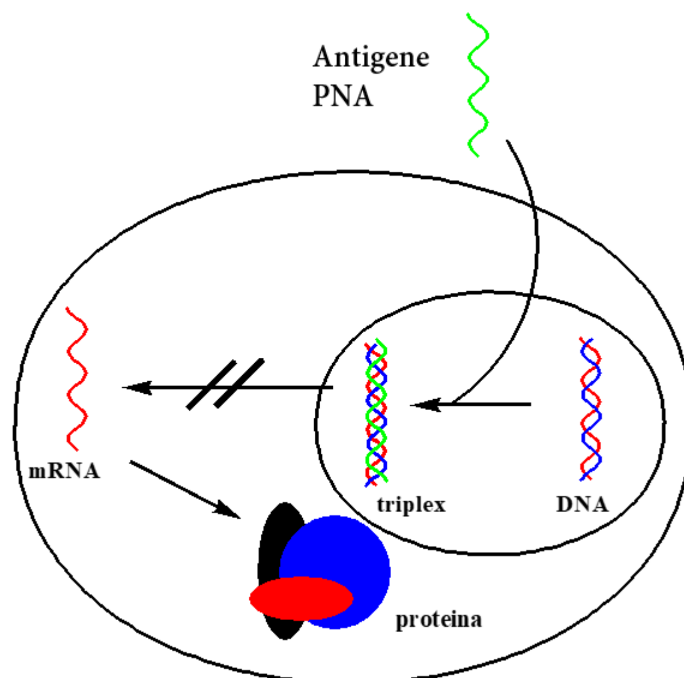

Figure 1. Schematic representation of the PNA-mediated antigen strategy. Specific binding of PNA molecules to complementary target DNA leads to suppression of gene transcription.

REFERENCES:

1. *Cesaro, E.; Lupo, A.; Rapuano, R.; Pastore, A.; Grosso, M.; Costanzo, P. (**2021**), Molecules 2021, 26, 6296.*
2. *Cesaro, E., Falanga, A. P., Catapano, R., Greco, F., Romano, S., Borbone, N., Pastore, A., Marzano, M., Chiurazzi, F., D'Errico, S., Piccialli, G., Oliviero, G., Costanzo, P., & Grosso, M. (**2022**). PloS one, 17(3), e0266090.*
3. *Greco, F.; Cesaro, E.; Falanga, A.P.; Catapano, R.; Romano, S.; Borbone, N.; Pastore, A.; Marzano, M.; D'errico, S.; Piccialli, G.; Oliviero, G.; Costanzo, P.; Grosso, M., (**2021**), in Proceedings of the 7th International Electronic Conference on Medicinal Chemistry, 1–30 November 2021, MDPI: Basel, Switzerland.*

The behaviour of nanocomposites containing vinyltrietoxysilane-grafted cellulose nanocrystals

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Vinyltrietoxysilane-grafted cellulose nanocrystals (VTES-CNCs) can act as a reinforcing filler improving mechanical, thermal, and optical properties of the material, leading to enhanced performance in applications such as coatings, wound dressings, and graft materials for biomedical applications. The behaviour of nanocomposites containing VTES-CNCs is influenced by several factors, including the type and amount of silane used for silanization, the size and distribution of the CNCs, and the type of polymer matrix. In this project, we evaluated the mechanical properties (flexural strength) and microbiological behaviour (activity against microbial adherence and biofilm formation) of light-curable methacrylate-based resin nanocomposites containing different concentrations of VTES-CNCs. Pristine resin served as a control. Flexural strength shows no significant decrease in mechanical properties when 5% VTES-CNCs was added. Preliminary data showed significant and broad-spectrum antiadhesive activity by CNCs against several microbial strains. Here, we show that VTES-CNC addition did not significantly change the microbiological behaviour of the nanocomposite. Further studies are currently underway to better ascertain the activity mechanism of VTES-CNC towards microbial cells.

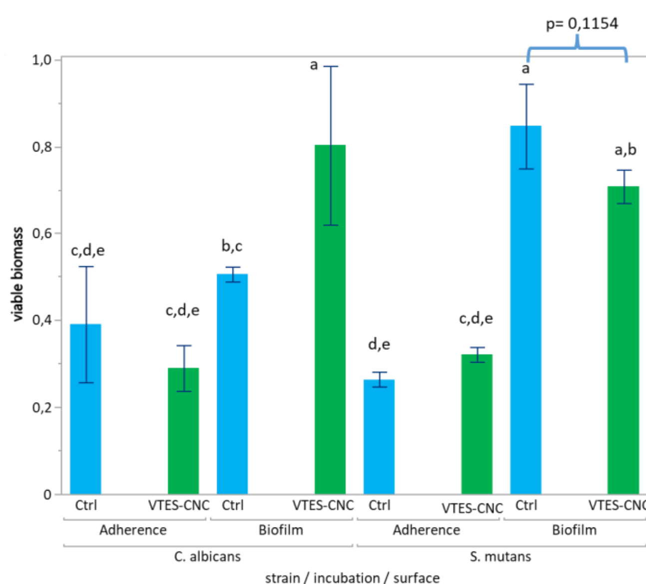

Figure 1. S. mutans and C. albicans adherent cells on the surface of the tested materials after 2 h, and biofilm formation after 24h incubation, as assessed by MTT test.

REFERENCES:

1. Ng, H.-M.; Sin, L. T.; Tee, T.-T.; Bee, S.-T.; Hui, D.; Low, C.-Y.; Rahmat, A. R. Composites Part B: Engineering 2015, 75, 176–200.
2. Miao, C.; Hamad, W. Y. Current Opinion in Solid State and Materials Science 2019, 23 (4), 100761.

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Designed copper metalloenzymes for the production of value-added chemicals

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In the recent years, waste materials have attracted academic and industrial communities aiming to identify strategies to convert waste to value-added materials and chemicals.[1] The reutilization of vegetable and industrial waste is therefore one of the main global challenges in mitigating environmental impact.
The exploitation of such waste chemicals (e.g. lignin, chitin, cellulose, phenol-like species) for value-added products is considered of remarkable scientific and social impact. In particular, the oxidative degradation of recalcitrant polymeric substrates for the conversion into second-generation biofuels, as catalyzed by bacterial Lytic Polysaccharide MonoOxygenases (LPMOs), and the conversion of toxic phenol-like species into value-added products, as performed by Polyphenol Oxidases (PPOs), may represent the first step for the valorization of waste chemicals.
Structural motif of the active site of LPMOs and PPOs, which both catalyzes the oxidation of exceedingly strong C-H bonds, are copper binding sites.[2] Reproducing the catalytic features of these enzymes in de novo designed protein scaffolds[3] can be important for structure-function relationship studies, but also for the development of efficient enzymes useful in waste treatment.
To this end, we adopted helix-bundle scaffolds to accommodate these copper binding sites. These simple models represent a milestone in the development of synthetic metalloenzymes for the degradation and conversion of biomass into second generation fuels.

Financial support by MUR (SEA-WAVE 2020BKK3W9) is gratefully acknowledged.

Figure 1. Engineered copper-binding sites involved in waste treatment.

REFERENCES:

1. Fairley, P. Introduction: Next generation biofuels. *Nature* **474**, S2–S5 (2011).
2. Solomon, E. I. *et al.* Copper Active Sites in Biology. *Chem. Rev.* **114**, 3659–3853 (2014).
3. Nastri, F. *et al.* Engineering Metalloprotein Functions in Designed and Native Scaffolds. *Trends Biochem. Sci.* **44**, 1022–1040 (2019).

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WORKSHOP: ***I chimici per le biotecnologie***

Napoli, 27 Febbraio 2023

Functionalization of cellulose nanocrystals for incorporation in resin composites of biomedical interest

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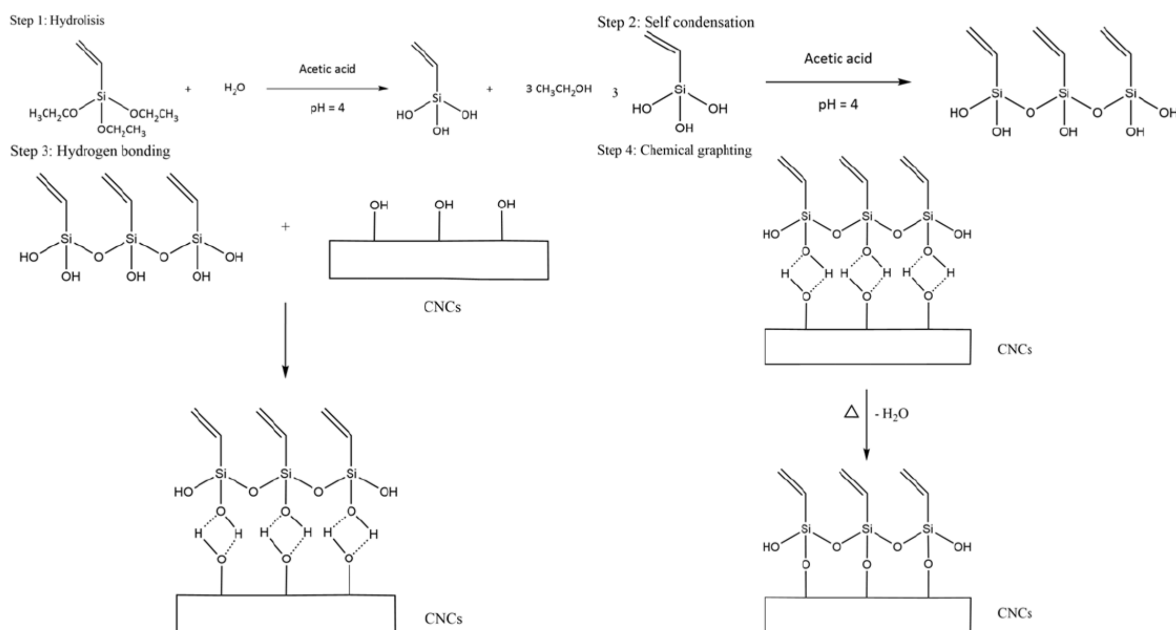
Cellulose nanocrystals (CNC) have gained increasing interest thanks to their wide availability, low cost (relative to other nanoparticles), renewability, and high strength[1,2]. Recent research showed very promising activity by CNCs against microbial adherence[2]. However, CNCs are too hydrophilic to be used as is in hydrophobic resin matrixes, since some agglomeration occurs. These nanoparticles allow the grafting of different functional groups onto their surface to facilitate further applications. This project aims to develop silanized CNC (SiCNC) for incorporation into methacrylate-based resin nanocomposites of biomedical interest. SiCNC were obtained by reaction of CNC with vinyltriethoxysilane (VTES, 2.5:1 to CNC), in an ethanol-water solution (1.5:1) in presence of acetic acid at pH=4.0, increasing temperature until 110 °C for 5 h (Scheme 1). SiCNC were extensively washed under ethanol, centrifuged, suspended in an aqueous solution, and lyophilized. SiCNC were characterized by ATR-FTIR, TGA, and water contact angle measurements. Non-functionalized CNCs served as control. Characterization showed the successful synthesis of Si-CNC with a 2.6g VTES/100g SiCNC and a hydrophobic nature. These materials are highly promising for the development of innovative biomedical nanocomposites with enhanced behaviour from mechanical, aesthetic, and microbiological/biological points of view. Fields of application may include materials for dental restorations, implantable stents, and grafts.



Scheme 1. Silanization reaction.

REFERENCES:

1. Eyley, S.; Thielemans, W. *Nanoscale* **2014**, *6* (14), 7764–7779.
2. Mali, P.; Sherje, A. P. *Carbohydrate Polymers* **2022**, *275*, 118668.

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WORKSHOP: *I chimici per le biotecnologie*

Napoli, 27 Febbraio 2023

Synthesis and characterization of nanomaterials based on halloysite modified with porphyrins for biological purposes

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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, focalizing 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.

REFERENCES:

1. Massaro, M.; Cavallaro, G.; Colletti, C. G.; Lazzara, G.; Milioto, S.; Noto, R.; Riela, S.; J. Mater. Chem. B, **2018**, 6, 3415-3433. 10.1039/C8TB00543E

Self-assembling nanoparticles for RNA delivery in personalized medicine

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RNA delivery represents one of the key tools for new therapeutic strategies. Messenger RNA (mRNA) can open new horizons in the treatment of diseases characterized by an altered protein expression. The recently approved RNA-based Covid vaccines have highlighted the potentialities of mRNA for vaccination, which can be leveraged to prevent viral infections and to treat cancer. Furthermore, since the discovery of RNA interference (RNAi), the knowledge on the pivotal role of non-coding RNA oligonucleotides such as small interfering RNA (siRNA) and microRNA (miRNA) in regulating cell processes boosted research for the use of these synthetic RNA fragments as novel drugs.

However, the development of RNA-based therapies is hampered by the poor biopharmaceutical profile of RNA, with rapid degradation in biological fluids and negligible uptake into the cells. Following the approval of Onpattro® and of the RNA-based vaccines Comirnaty® and Spikevax®, lipid nanoparticles (LNPs) are considered the leading solution to develop RNA-based therapeutics. However, despite the success of lipid nanoparticles (LNP), some issues still need to be addressed. The poor physical stability of RNA-loaded LNP requires low temperatures for storage and transport and the industrial scale-up of the formulations developed at lab scale remains a challenge.

To address these issues, our research group developed a novel nanotechnology approach named lipid self-assembling nanoparticle (SANP) as an alternative platform for RNA delivery. Lipid SANP formulations have shown remarkable biocompatibility, high RNA encapsulation efficiency, and enhanced intracellular release. Furthermore, SANP have been designed to be prepared at room temperature immediately before use by simple mixing three components, a calcium/phosphate dispersion, RNA, and cationic liposomes. By using this approach, the RNA can be stored and used in a lyophilized form, which ensures greater stability against degradation compared to freezing. The SANP technology is well-suited for the development of personalized nanomedicines since it is possible to tune the type and amount of RNA to be administered on a single patient base.

SANPs have been proposed to deliver miRNA [1] or siRNA [2] for the treatment of different tumors and we are currently investigating SANPs encapsulating mRNA for vaccination purposes. Further development of the SANP platform includes the inclusion of “bioactive” components into the nanoparticle core able to prevent neuron damage following oxidative stress, thus making this technology of interest for the treatment of neurodegenerative diseases [3].

REFERENCES

[1] Campani V, et al. Int J Pharm. 2020 Oct 15;588:119693.

[2] Delle Donne et al. Commun Biol. 2022 Aug 2;5(1):780.

[3] Nele V., et al. Antioxidants 2023, 12(2), 358.

Società Chimica Italiana
Gruppo Interdivisionale Biotecnologie

WORKSHOP: *I chimici per le biotecnologie*

Napoli, 27 Febbraio 2023

Self-Assembly of new G-Rich Oligonucleotides Incorporating a 3'–3' Inversion of Polarity Site

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The development of new DNA-based biomaterials is very important in the field of nanotechnology and represents one of the most interesting challenges in the scientific world. Previous studies have shown that DNA can adopt specific structures that act as building blocks for the assembly of supramolecular structures [1]. Among the DNA secondary structures there is the G-Quadruplex, which is formed by selected guanosine-rich sequences and is characterized by the presence of structural units called G-quartets consisting in planar arrangements of four guanines held together by eight Hoogsteen type hydrogen bonds. In the G-quadruplexes, two or more G-quartets are stacked one on top of the other by π-π hydrophobic interactions and the resulting structure is stabilized by monovalent cations located in the central cavity of the G-quadruplex axis. The combination of π-π stacking interactions and coordination with cations render G-quadruplexes by far more stable than DNA duplexes of the same length [2]. In this study, we report an innovative strategy to obtain a new type of DNA G-wire nanomaterial, called Q_n, starting from the short unmodified G-rich oligonucleotide d(5'-CGGT-3'–3'-GGC-5') (1), exhibiting a 3'–3' inversion of polarity site. The inversion of the polarity site was achieved by performing the first four coupling cycles with 5'-phosphoramidites and the remaining three with standard 3'-phosphoramidites. The "n" subscript in Q_n indicates the number of tetramolecular G-quadruplex building blocks involved in the G-wires elongation. The described building block can multimerize in a stacked G-Wire polymer through 5'–5' π-π stacking interactions [3][4]. The effect of the presence of polar or lipophilic groups at the edges of the G-rich ODNs on the topology and stability of resulting G-quadruplexes is also under study in our laboratories.

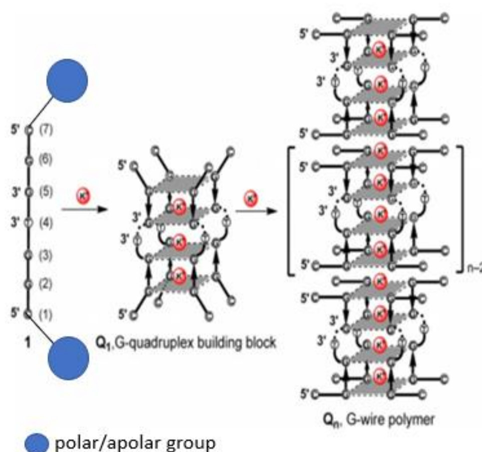

Figure 1. Formation of the G-quadruplex building block Q_1 and its multimerization into Q_n G-wire polymers starting from the ODN 1. Polar/apolar groups are shown as blue spheres.

REFERNCES

1. Ozin, G.A.; Hou, K.; Lotsch, B. v.; Cademartiri, L.; Puzzo, D.P.; Scotognella, F.; Ghadimi, A.; Thomson, J. Nanofabrication by Self-Assembly. *Materials Today* 2009, *12*, 12–23.
2. Collie, G.W.; Parkinson, G.N. The Application of DNA and RNA G-Quadruplexes to Therapeutic Medicines. *Chem Soc Rev* **2011**, *40*, 5867–5892, doi:10.1039/c1cs15067g.
3. Oliviero, G.; D'Errico, S.; Pinto, B.; Nici, F.; Dardano, P.; Rea, I.; de Stefano, L.; Mayol, L.; Piccialli, G.; Borbone, N. Self-Assembly of g-Rich Oligonucleotides Incorporating a 3'-3' Inversion of Polarity Site: A New Route towards G-Wire DNA Nanostructures. *ChemistryOpen* **2017**, *6*, 599–605, doi:10.1002/open.201700024.
4. Borbone, N.; Amato, J.; Oliviero, G.; D'Atri, V.; Gabelica, V.; de Pauw, E.; Piccialli, G.; Mayol, L. D(CGGTGGT) Forms an Octameric Parallel G-Quadruplex via Stacking of Unusual G(:C):G(:C):G(:C):G(:C) Octads. *Nucleic Acids Res* **2011**, *39*, 7848–7857, doi:10.1093/nar/gkr489.

An innovative approach for OC extraction from bone powder

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The most abundant non-collagen protein in bones and an important component of the extracellular bone matrix is osteocalcin (OC), a representative bone γ-carboxyglutamic acid (Gla) protein (BGP) (1). Human Osteocalcin (hOC) is produced in osteoblast as a pre-pro-peptide, composed of 49 amino acids and undergoes several post-translational modifications before its secretion (2). The most important modification is carboxylation of glutamic acid residues (Glu) at position 17, 21 and 24 by a vitamin-K-dependent γ-glutamyl carboxylase (GGCX). The carboxylation leads to a greater affinity for Ca^{2+} ions present in bone matrix and involved in the formation of a high-affinity mineral-protein complex. This effect is the result of the particular configuration of carboxylation: –COOH group linked in γ of selected glutamate residues (Glu→Gla) builds a specific site composes by two carboxylic groups that can bind simultaneously a Ca^{2+} ions in the bones (5). These interactions are responsible for a conformational transition from an unstructured random coil to a folded protein and they are a hard link between protein and inorganic matrix (1). The undercarboxylated OC cannot bind in the bone matrix and it leaks into the blood where it carries out a role as metabolic hormone (3,4). The interactions with Ca^{2+} ions make it difficult to recover OC by bone without having by-products like calcium salt.

Due to the great interest in hOC as a diagnostic marker, it could be useful to have a specific and efficient way to extract OC from bone matrix. There are several strategies reported in literature, such as bone powder demineralization with acids, or the use of chelating agents able to bring out Ca^{2+} ions in order to have a more accessible proteome. Moreover, there are some possibilities that combine chemical and biochemical techniques, but it is necessary to identify one method that can extract OC with specificity and efficiency. In line with these considerations, we have identified oxalate as the agent for OC extraction from bone powder and applied two different approaches: directed extraction or demineralization followed extraction. The force point of oxalate is that it can bind Ca^{2+} ions with higher affinity than OC, forming the salt calcium oxalate, with a very low solubility in water. In direct extraction we used ammonium oxalate to bone powder, while in the second option we preferred to use chelating agents like ethylenediaminetetraacetic acid (EDTA) or oxalic acid for demineralization following extraction with ammonium oxalate. Our results showed that all these experimental approaches can extract OC with similar yield; however, they all required a dialysis step to completely remove the saline component. In the future prospective we would like to test citric acid as both acid and chelating agent.

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REFERNCES

1. **Li J, Zhang H, Yang C, Li Y, Dai Z. J Bone Miner Metab.** 2016 Jul;34(4):367-79.
2. Zoch ML, Clemens TL, Riddle RC. Bone. 2016 Jan; 82:42-9.
3. Kapoor, K., Pi, M., Nishimoto, S. K., Quarles, L. D., Baudry, J., & Smith, J. C. (2021). Biochimica et Biophysica Acta - General Subjects, 1865(3).
4. Price PA, Nishimoto SK (1980). Proc Natl Acad Sci USA 77:2234–2238.
5. Burnier, John P., et al. Molecular and Cellular Biochemistry 39 (1981): 191-207.

Development of different protocols for ketoreductase immobilization: application in asymmetric reduction of a ketone in a flow system

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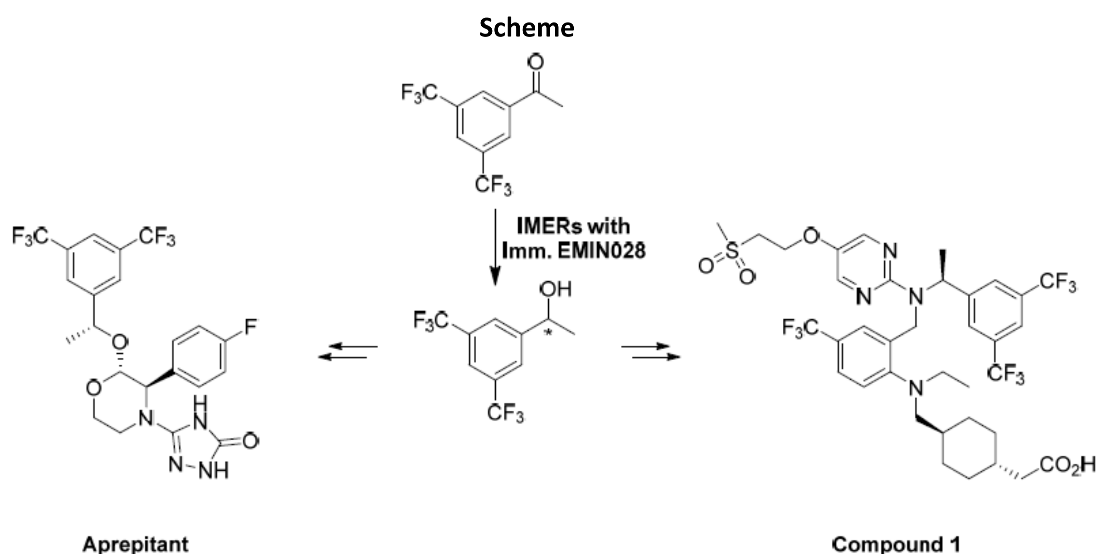
Biocatalytic reduction of prochiral ketones to chiral alcohols has emerged as an important tool for the synthesis of active pharmaceutical ingredients (APIs).[1] Many ketoreductases (KREDs) are now commercially available and their efficient application has been demonstrated also on industrial scale.[2] However, the use of these enzymes is often hampered by low operational stability due to inactivation from organic solvents, reagents and products and to difficulties in recovery and reuse of the biocatalyst. In recent years, immobilization of KREDs has been investigated in order to obtain stable and recyclable biocatalysts.[3] In this work, the immobilization of a commercial KRED (*EMIN028* from Enzymaster) was studied on a set of supports with different features in terms of functional groups (epoxy, amino) and enzyme-carrier interactions (covalent, ionic, hydrophobic). The immobilized enzyme samples were employed for the synthesis of (*S*)-1-(3,5-bis(trifluoromethyl)phenyl)ethanol, an important chiral intermediate for the preparation of NK-1 receptor antagonist Aprepitant and of Compound 1, which has been proved to be active in treating or preventing immunodiseases (Scheme). Biotransformations were run in packed bed reactors (IMERs) in a flow system using 90% IPA/10% (buffer or water) solvent system. Several consecutive reactions were performed, obtaining high yields and >99% ee in 24 hours cycles. The obtained results will be shown and discussed.

Scheme



REFERNCES

1. Moore, J.C.; Pollard, D.J.; Kosjek, B.; Devine, P.N. *Acc. Chem. Res.* **2007**, *40*, 1412–1419.
2. (a) Wu K, Zheng K, Xiong L, Yang Z, Jiang Z, Meng X, S. L. *Appl Microbiol Biotechnol* **2019**, *103*, 4417–4427; (b) Zong, C.; Zhang, X.; Yang, F.; Zhou, Y.; Chen, N.; Yang, Z.; Ding, G.; Yu, F.; Tang, Y. *Prep. Biochem. Biotechnol.* **2019**, *49*, 578–583.
3. (a) Adebar, N.; Gröger, H. *Bioengineering* **2019**, *6*, 99; (b)Phipps, J.; Chen, H.; Donovan, C.; Dominguez, D.; Morgan, S.; Weidman, B.; Fan, C.; Fan, C.; Beyzavi, M. H.; Beyzavi, M. H. *ACS Appl. Mater. Interfaces* **2020**, *12*, 26084–26094

Fe(III)-MimochromeVI*a for the development of sensitive lateral flow immunoassays

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Lateral flow immunoassays (LFiAs) are point-of-care tests commonly used around the world thanks to their reliability, sensitivity, and specificity. When the output of the assay is a colorimetric signal, gold nanoparticles (AuNPs) are often used because of their high surface-to-volume ratio and naked-eye visible red color (when 20 nm in diameter, as is most commonly used).[1] Despite many advantages, the LFiAs lack the sensitivity required to detect many desirable biomarkers, which currently have to be detected with laboratory assays. Recent studies have therefore focused on developing signal enhancement strategies, including the use of enzyme catalyzed reactions.[2]

Herein, the artificial miniaturized peroxidase Fe(III)-MimochromeVI*a (FeMC6*a) is exploited as a strategy to obtain catalytic signal amplification in sandwich immunoassays on lateral flow strips. The here developed Human-IgG assay foresees the use of AuNPs decorated with both FeMC6*a and anti-Human-IgG as a detection antibody. After AuNPs focusing over the test line, subsequent addition and catalytic oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by FeMC6*a induces an increase of the test line color (Figure 1).

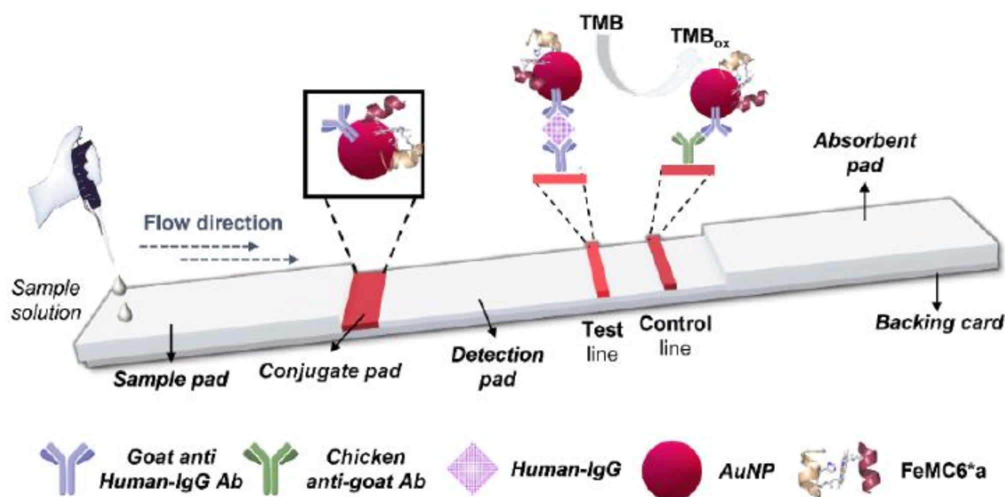

Figure 1. *Schematic representation (not to scale) of FeMC6*a –assisted lateral flow two-step immunoassay for Human-IgG detection.*

Our results show that FeMC6*a acts as an efficient catalyst on paper, increasing the sensitivity of LFiA up to 4 times with respect to a conventional LFiA. Furthermore, FeMC6*a achieved lower limits of detection that were found in control experiments horseradish peroxidase, its natural counterpart. This study represents a significant proof-of-concept for the development of more sensitive LFiAs, for different analytes, based on properly designed artificial metalloenzymes.

REFERNCES:

1. Parolo, C., Sena-Torralba, A., Bergua, J. F., Calucho, E., Fuentes-Chust, C., Hu, L., Rivas, L., Álvarez-Diduk, R., Nguyen, E. P., Cinti, S., Quesada-González, D., Merkoçi, A. *Nat. Protoc.* **2020**, *15*, 3788-3816.
2. Calabria, D., Calabretta, M. M., Zangheri, M., Marchegiani, E., Trozzi, I., Guardigli, M., Michelini, E., Di Nardo, F., Anfossi, L., Baggiani, C., Mirasoli, M. *Sensors* **2021**, *21* (10), 3358-3377.

Synthesis and characterization of PNA-human ferritin shuttles

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Peptide Nucleic Acid (PNA) has attracted great attention as valuable antisense therapeutic molecule[2]. However, both the poor cell permeability and solubility remain challenges for biomedical application. To overcome these drawbacks, PNA needs to be delivered by using a carrier system. In this work the internal cavity of "humanized" chimeric Archaeal ferritin (HumAfFt)[1] specifically decorated with cationic piperazine-based compounds (PAs), was exploited to load and transport antisense PNA within cancer cell lines. PNA 10-mer was designed complementary to a tract of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, whose a housekeeping gene is implicated in the catalysis of an important energy step in carbohydrate metabolism. PNA probe was synthesized with a negative glutamic acid peptide tail, to promote electrostatic interaction with the positive polyamines of the piperazine-based compounds in the inner cavity of HumAfFt (Figure 1). To this aim two PNAs were synthesized bearing 4 or 8 glutamic acid residues to investigate the loading efficiency of the carrier. In addition, to study the capability of the HumAfFt nanocarrier to deliver PNA molecules into cells, the sequences were covalently modified by fluorescein 5-isothiocyanate (FITC) probe for uptake studies in HeLa, HepG2, and MCF-7 cancer cell lines.

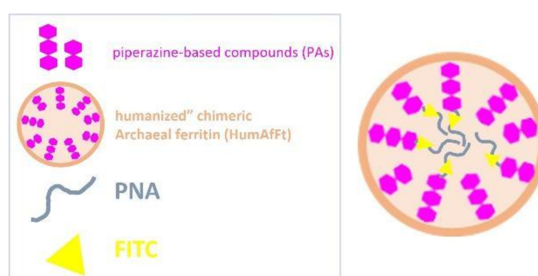

Figure 1: Schematic representation of HumAfFt-PNA.

REFERNCES

1. Pediconi, Natalia, et al. "Design and synthesis of piperazine-based compounds conjugated to humanized ferritin as delivery system of siRNA in cancer cells." *Bioconjugate Chemistry* 32.6 (2021): 1105-1116.
2. Karkare, S., Bhatnagar, D. Promising nucleic acid analogs and mimics: characteristic features and applications of PNA, LNA, and morpholino. *Appl Microbiol Biotechnol* **71**, (2006): 575–586

Controlled release of gallic acid from cyclodextrin-based nanosponges

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Bioactive compounds such as vitamins, essential oils, fatty acids, flavors, aroma components, and phenolic compounds, are crucial for human health. Gallic Acid (GA) is a polyphenol increasingly used in the pharmaceutical industry due to its antioxidant, anti-inflammatory, antibacterial, neuroprotective and antitumor properties, and for food preservation, due to its ability to reduce the rancidity.
However, various factors limit the application of these molecules in food, including low stability due to sensitivity to oxygen, light and temperature, poor solubility, and low bioavailability[1]. The use of encapsulation agents for active compounds is a strategy that allows to increase their solubility, bioavailability and stability. Another important advantage of encapsulation is the controlled release of bioactive compounds.
Among encapsulation processes, spray-drying, spray-cooling and extrusion are employed in industrial processes, but only molecular inclusion occurs at molecular level and, thus, one molecule of the active compound is trapped in the cavity of the host molecule[2]. The union between the active compound and the host molecule is called inclusion complex. One of these host molecules is cyclodextrin, since the Food and Agriculture Organization (FAO) of the United Nations recognizes it as additive. Cyclodextrins (CDs) are amazing molecules because of their peculiar and amphiphilic structure. Since the glucopyranose units are in the chair conformation, CDs have the form of a truncated cone or torus with a hydrophobic cavity. CDs can easily form reticulated structures by co-polymerization with appropriate crosslinkers. Among the most used crosslinkers, epichlorohydrin (EPI) originates a hydrophilic gel with CD monomers connected by repeating glyceryl units. The properties of cyclodextrins allow the development of innovative materials such as NanoSponges (NSs), versatile supramolecular 3D–hyperreticulated materials, with high surface area and thermal stability, and extraordinary ability to adsorb compounds with different degrees of hydrophilicity/hydrophobicity[3].
The aim of the present study was to obtain and characterize cyclodextrin-based nanosponges loaded with gallic acid for potential applications in the agrifood field. Chemical (1H-NMR, UV vis-spectra), morphological (Scanning Electron Microscopy, SEM) and biological (antioxidant activity) analyses were carried out. Furthermore, the release results obtained as function of pH and time showed that CDNSs are good candidate for applications in the food industry.

REFERNCES

1. Assadpour, E.; Jafari, S.M. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 3129-3151.
2. Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. *J. Food Sci.* **2002**, *67*, 3271-3279.
3. Utzeri, G.; Matias, P.M.C.; Murtinho, D.; Valente, A.J.M. Front. Chem. **2022**, *10*, 859406-859430.

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Metal site geometry and coordination number prediction with neural networks

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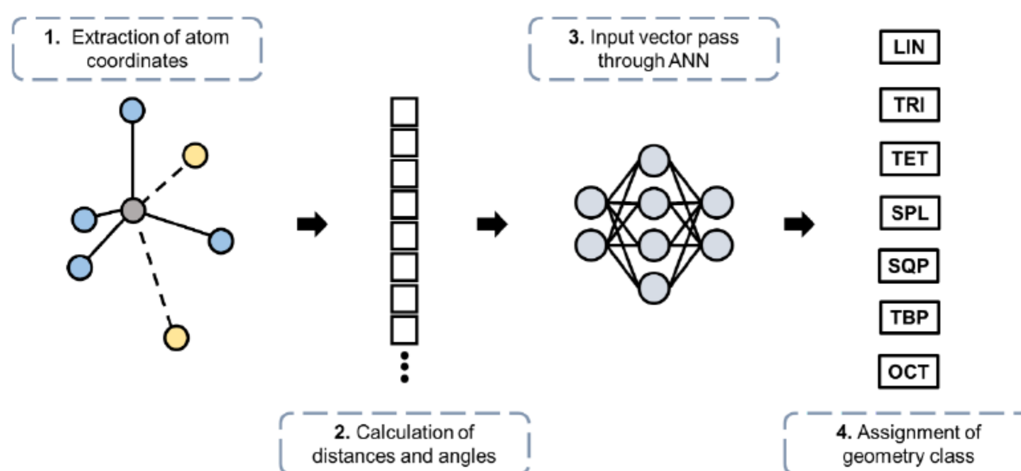
The chemical properties of metal complexes, both abiotic and biological, are significantly impacted by their coordination number and geometry. An automated method for determination of these features from atomic coordinates would be useful in areas such as structure quality control, data-mining, and machine learning. However, conventional methods for this task tend to require significant computational resources. To address this, we present a deep learning-based approach using artificial neural networks (ANNs) to simultaneously classify coordination number and geometry [1]. Crystal structures of metal complexes with a coordination number ranging from 2 to 6 in seven prevalent coordination geometries were obtained from the CSD and MetalPDB databases [2,3]. The geometric features, such as the distances and angles formed by the six closest atoms to the metal, were calculated from crystallographic coordinates. These features were then utilized as input data to train separate models for classification of abiotic and biomolecular sites (Scheme 1). After hyperparameter optimization, the ANN trained on structures deposited in the CSD reaches 96.5% balanced accuracy on classification of small-molecule metal complexes in the validation dataset. Surprisingly, the performance of this model on PDB deposited metal sites is also remarkable with 95.7% balanced accuracy for a manually reviewed PDB validation set, showing a notable degree of generalization by the ANN. Overall, these results demonstrate the validity of this approach for automated metal site classification, in particular for high-throughput applications which require the extraction of molecular properties from a large number of structures.



Scheme 1: General pipeline for input vector generation and classification through ANN.

REFERENCE:

1. A. Krogh, Nat. Biotechnol. **2008**, 26, 195–197.
2. C. R. Groom, I. J. Bruno, M. P. Lightfoot and S. C. Ward, Acta Cryst. **2016**, B72, 171-179
3. V. Putignano, A. Rosato, L. Banci and C. Andreini, Nucleic Acids Res. **2018**, 46(D1):D459-D464.

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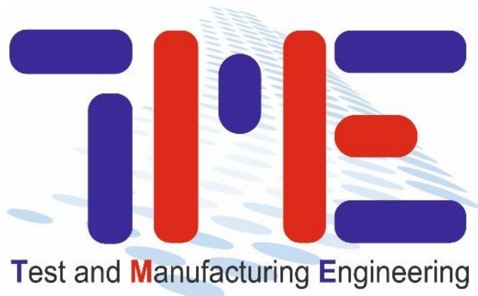

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